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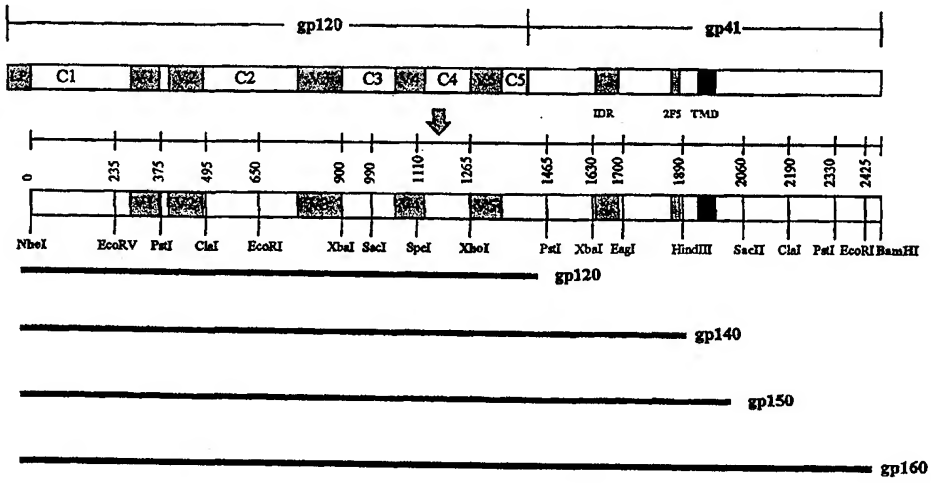
(54) Title: METHOD FOR PRODUCING A NUCLEOTIDE CONSTRUCT WITH OPTIMISED CODONS FOR AN HIV GENETIC VACCINE BASED ON A PRIMARY, EARLY HIV ISOLATE AND SYNTHETIC ENVELOPE BX08 CONSTRUCTS

Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms



(57) Abstract

The present invention relates to a method for producing a nucleotide sequence construct with optimized codons for an HIV genetic vaccine based on a primary, early HIV isolate. Specific such nucleotide sequence construct are the synthetic envelope BX08 constructs. The invention further relates to the medical use of such constructs for the treatment and prophylaxis of HIV through DNA vaccine and for diagnostics.

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Method for producing a nucleotide sequence construct with optimised codons for an HIV genetic vaccine based on a primary, early HIV isolate and synthetic envelope BX08 constructs.

Field of the invention

The invention relates to a DNA vaccine against HIV, which is designed from a clinical primary isolate. One aspect of the invention relates to a method of producing a nucleotide sequence construct, in a prefered aspect based on a cassette system, the nucleotide sequence construct being used as a DNA vaccine. The method can, for example, lead to the disclosed synthetic BX08 HIV-1 envelope vaccine nucleotide sequence construct, designed to generate suitable DNA vaccines against HIV, specifically HIV-1. Furthermore, the invention can be used for the production of recombinant protein antigens.

Background of the invention

There is an urgent need for new vaccine strategies against HIV. One such new promising strategy is called genetic immunisation or DNA vaccine (Webster et al 1997). Some of the advantages of a DNA vaccine against HIV is the induction of Th cell activation, induction of antibodies also against conformational dependent epitopes, and the induction of cellular immunity. So far, most DNA vaccine envelope genes tried, have been from tissue culture adapted virus strains (Boyer et al 1997) that often differs in several aspects from primary clinical isolates (such as early isolates) e.g. in co-receptor usage (Choe et al 1996, Dragic et al 1997).

One disadvantage in HIV envelope based DNA vaccines may be the intrinsic relatively low expression which is regulated by the Rev expression. This may prevent an optimal investigation of the vaccines in small animal models like mice where Rev is functioning suboptimally. Recently it has been shown using the tissue culture adapted HIV-1 MN strain, that an exchange of the HIV codon usage to that of highly expressed mammalian genes greatly improves the expression in mammalian cell lines and renders the HIV expression Rev independent (Haas et al 1996). Additionally, it is known that rare codons cause pausing of the ribosome, which leads to a failure in completing the nascent polypeptide chain and a uncoupling of transcription and translation. Pausing of the ribosome is thought to lead to exposure of the 3' end of the mRNA to cellular ribonucleases.

The world-wide spread of HIV-1 has presently resulted in 8,500 new infections daily and AIDS is now the number 1 cause of death among US males (and number 3 among US females) aged 25-40 years. The epidemic hot-spots now include Eastern Europe, India and South East Asia and southern Africa. The attempts to solve this world-wide problem involve education, prevention, treatment and vaccine development. Affordable protective vaccines represent the best solution to the world-wide problem of infection with HIV-1. Induction of virus neutralising antibodies is one of the key components in vaccine development. Several recombinant envelope vaccines have been tested in humans and animals, however, they seem unable to induce sufficient protection. In this respect DNA vaccination may provide a different and more natural mode of antigen presentation. It is hoped that the immune responses induced by such DNA vaccines could aid in limiting virus replication, slowing disease progression or preventing occurrence of disease. Unfortunately many HIV envelope vaccines induce only moderate levels of antibodies. This could in part be due to limitations in expression, influenced by regulation by the Rev protein and by a species-specific and biased HIV codon usage. Also the virus variability is considered a barrier for development of antibody based vaccines and thus a tool for more easy producing of closely related vaccine variants is needed.

It has been suggested to improve the immunogenicity and antigenicity of epitopes by certain mutations in the envelope gene. An elimination of certain immune dominant epitopes (like V3) could render less immune dominant but more relevant, conserved, or hidden epitopes more immunogenic (Bryder et al 1999). Also elimination of certain N-linked glycosylation sites could improve the exposure of relevant epitopes and increase the immunogenicity of those epitopes. Thus, it is possible that elimination of the glycosylation sites in V1 and V2 may in a more favourable way expose neutralising epitopes (Kwong et al 1998, Wyatt et al 1998). The HIV envelope contains putative internalisation sequences in the intracellular part of gp41 (Sauter et al 1996). Thus it would be relevant to eliminate and/or mutate the internalisation signals in a membrane bound HIV envelope vaccine gene to increase the amount of surface exposed vaccine derived HIV glycoproteins as gp150. Since the antibody response, that is measured and calculated in titers, is improved by adding the secreted gp120 as opposed to adding the membrane bound form (Vinner et al 1999), it could be advantageous to express the vaccine as a secreted gp120 or a secreted gp140. This would include important parts of gp41, such as the 2F5 neutralising linear epitope (Mascola et al 1997).

Summary of the invention

Our suggested solution to the problems described above is to design DNA envelope vaccines from a clinical primary isolate with Rev-independent high expression in mammals, that is built as a cassette for easy variant vaccine production.

A method of producing a nucleotide sequence construct with codons from highly expressed mammalian proteins based on a cassette system coding for an early, primary HIV envelope is described. The method comprises the steps of direct cloning of an HIV gene, derived from a patient within the first 12 months of infection, thereby obtaining a first nucleotide sequence; designing a second nucleotide sequence utilising the most frequent codons from mammalian highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence; redesigning the second nucleotide sequence so that restriction enzyme sites surround the regions of the nucleotide sequence encoding functional regions of the amino acid sequence and so that selected restriction enzyme sites are removed, thereby obtaining a third nucleotide sequence encoding the same amino acid sequence as the first and the second nucleotide sequence; redesigning the third nucleotide sequence so that the terminals contain convenient restriction enzyme sites for cloning into an expression vehicle; producing snuts between restriction enzyme sites as well as terminal snuts and introducing snuts into an expression vehicle by ligation. The nucleotide sequence construct obtained by this method uses the mammalian highly expressed codons (figure 1) and renders the envelope gene expression Rev independent and allows easy cassette exchange of regions surrounded by restriction enzyme sites that are important for immunogenicity, function, and expression.

The method can, for example, lead to the disclosed synthetic, Rev-independent, clinical (such as early), primary HIV-1 envelope vaccine gene, built as a multi cassette. From the sequence of the envelope of the HIV-1 BX08 isolate (personal communication from Marc Girard, Institute Pasteur, Paris), the present inventors have designed a synthetic BX08 HIV-1 envelope vaccine nucleotide sequence construct.

With the great diversity of envelopes in HIV among different patients and within one patient, it would be of advantage to vaccinate with several envelope variants, all being highly expressed. To avoid synthesising several full length envelopes, it is much easier to exchange relevant parts of an envelope cassette to various strains in a multivalent vaccine.

Whether it is the disclosed synthetic BX08 nucleotide sequence construct, or any of the nucleotide sequence constructs obtained by the method, they are designed to generate suitable DNA vaccines against HIV, specifically HIV-1. In this case the mammal, preferably a human being, is inoculated with the nucleotide sequence construct in an expression vehicle and constitutes a host for the transcription and translation of the nucleotide sequence construct. The nucleotide sequence constructs of the present invention can furthermore be used for the production of recombinant protein antigens. In this case the nucleotide sequence construct is placed in an expression vehicle and introduced into a system (e.g. a cell-line), allowing production of a recombinant protein with the same amino acid sequence. The recombinant protein is then isolated and administered to the mammal, preferably a human being. The immune system of the mammal will then direct antibodies against epitopes on the recombinant protein. The mammal, preferably a human being, can thus be primed or boosted with DNA and/or recombinant protein obtained by the method of the invention.

A relevant HIV DNA vaccine can potentially be used not only as a prophylactic vaccine, but also as a therapeutic vaccine in HIV infected patients, e.g. during antiviral therapy. An HIV specific DNA vaccine will have the possibility to induce or re-induce the wanted specific immunity and help the antiviral therapy in limiting or even eliminating the HIV infection. The immunogenicity and antigenicity of epitopes in the envelope can be improved by certain mutations in the envelope gene. The cassette system allows for easy access to the relevant parts of the envelope gene, and thereby eased efforts in the process of genetic manipulation. Some suggested mutations are: an elimination of certain immune dominant epitopes (like V3); elimination of certain N-linked glycosylation sites (like glycosylation sites around V2); elimination and/or mutation of the nucleotide sequence encoding the internalisation signals in the cytoplasmic part of a membrane bound HIV envelope to increase the amount of surface exposed vaccine derived HIV glycoproteins; elimination or mutation of the cleavage site between gp120 and gp41; with introduced mutations in gp41 for preserving conformational epitopes.

Table 1 below, lists the nucleotide sequence constructs of the invention by the names used herein, as well as by reference to relevant SEQ ID NOs of DNA sequences, and the amino acid sequence encoded by the DNA sequence in the preferred reading frame. It should be noted, that the snut name consist of the number of the approximate position for the end of the snut and the restriction enzyme used to cleave and/or ligate that end of the snut.

Table 1 List of names of nucleotide sequence constructs (Snuts (S) and Pieces (P)) with reference to SEQ ID NO for the nucleotide sequence and protein sequence.

Name	Nucleotide SEQ ID NO:	Protein SEQ ID NO:
$S_{O\text{-}N\text{-}Lang}$	1	2
$S_{235EcoRV}$	3	4
$S_{375PstI}$	5	6
$S_{495ClaI}$	7	8
$S_{650\text{-}720EcoRI}$	9	10
$S_{900XbaI}$	11	12
$S_{990SacI}$	13	14
$S_{1110SpeI}$	15	16
$S_{1265XhoI}$	17	18
$S_{1265gp120}$	19	20
$S_{1265gp160}$	21	22
$S_{1465PstI}$	23	24
$S_{1465PstI\ cys}$	25	26
$S_{1630XbaI}$	27	28
$S_{1700EagI}$	29	30
$S_{1890HindIII}$	31	32
$S_{2060SacII}$	33	34
$S_{2190ClaI}$	35	36
$S_{2330PstI}$	37	38
S_{2425ES}	39	40
P_1	41	42
P_2	43	44
P_3	45	46
P_{3GV1}	47	48
$P_{3\ GV1V2}$	49	50
P_{3GV2}	51	52
P_{4gp160}	53	54
P_{4gp150}	55	56
P_{4gp140}	57	58
P_5	59	60
P_{8gp160}	61	62
P_{8gp150}	63	64
P_{8gp140}	65	66
synBX08-140	67	68
synBX08-150	69	70
synBX08-160	71	72
synBX08-120	73	74
synBX08-41	75	76

Detailed disclosure of the invention

One aspect of the present invention relates to a method for producing a nucleotide sequence construct coding for an HIV gene. The nucleotide sequence construct is produced as a cassette system consisting of snuts. A snut (S) is a nucleotide sequences construct between restriction enzyme cleavage sites comprising the minimal entity of the cassette system.

First an HIV gene is obtained from a patient within the first 12 months of infection. The term HIV should be understood in the broadest sense and include HIV 1 and HIV 2. It is possible to determine the period in which the infection has taken place with an accuracy depending on the frequency of the blood tests taken from the patient. For example, patients suffering from various diseases such as lack of certain factors in their blood or hepatitis have their blood tested on a regular basis making it possible to determine the period in which the infection has taken place. Apart from patients with diseases wherein blood tests are used to monitor the course of the disease, other groups of patients have blood tests taken, e.g. blood donors. Unfortunately, humans are still infected due to transfer of virus in blood samples, medical equipment, etc., making it possible to determine the date where the infection has taken place within the time frame of a few days. The importance of obtaining the virus early in the course of the infection is due to the known fact that many early isolates share the common feature of staying relatively constant in their envelope sequences (Karlsson et al., 1998). As these early isolates may share cross-reactive antibody- and/or T-cell epitopes a vaccine based on such early isolates would have a better chance of inducing immune response to shared epitopes of the virus. It is believed that an early, directly cloned virus isolate will share immunogenic sites with other early virus isolates seen during an HIV infection, so that if a mammal generates antibodies and/or T-cells directed against these epitopes, the transferred virus will be eliminated prior to the extensive mutations that may occur after approximately 12 months of infection. Thus, the virus should be isolated as early as possible, that is within the first 12 months of infection, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0.5 month after infection.

The HIV gene for genetic vaccine is preferably cloned directly from viral RNA or from proviral DNA. Direct cloning in this application stands for the virus not being multiplied in stable cell lines *in vitro*. It is presently expected that passing the virus through a stable cell line will promote mutation in the virus gene. It is particularly preferred not to pass the virus through cells lines selecting for viruses with CXCR4 receptor usage. Direct cloning also includes multiplication of virus in e.g. PBMC (peripheral blood mononuclear cells) since all virus can

multiply in PBMC, and this type of multiplication generally does not select for CXCR4 receptor usage. Multiplication of virus is often necessary prior to cloning. Preferably cloning is performed directly on samples from the patient. In one embodiment of the invention, cloning is performed from patient serum. The cloning is then performed directly on the HIV virus, that is on RNA. In another embodiment of the invention cloning, is performed from infected cells. The cloning is then performed on HIV virus incorporated in the genes in an infected cell (e.g. a lymphocyte), that is on DNA. In the latter case the virus might be a silent virus, that is a non-replicating virus. To evaluate if the virus is silent, capability of multiplication in e.g. PBMC is tested.

Cloning is a technique well known to a person skilled in the art. A first nucleotide sequence is hereby obtained. In another aspect of the invention, the first nucleotide sequence, sharing the properties mentioned with direct cloning, is obtained by other means. This could be from a database of primary isolates or the like.

Based on the first nucleotide sequence, the amino acid sequence encoded by said nucleotide sequence is determined. A second nucleotide sequence encoding the same amino acid sequence is then designed utilising the most frequent codons from highly expressed proteins in mammalians (e.g. figure 1 presenting the most frequent codons from highly expressed proteins in humans).

Presently, it appears that the usage of the most frequent codons from mammalian highly expressed proteins has two advantages: 1) the expression is Rev independent; 2) the level of expression is high. The Rev independence is especially advantageous when performing experiments in mice where the Rev systems is functioning sub-optimally. For the use in human vaccine, Rev independence and high expression are important to increase the amount of antigen produced. The determination of the codons for high expression is in this context based on the statistics from human highly expressed proteins (Haas, Park and Seed, 1996 hereby incorporated by reference). It is contemplated that the expression of a protein can be even higher, when current research in binding between codon (on the mRNA) and anticodon (on the tRNA) reveals codons with optimal binding capabilities, and when interactions in-between codons and/or in-between anticodons are known.

The second nucleotide sequence designed utilising optimised codons is then redesigned to obtain a third nucleotide sequence. The purpose of the redesigning is to create unique restriction enzyme sites around the nucleotide sequence encoding functional regions of the

amino acid sequence. By having unique restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence, the nucleotide sequence encoding functional regions of the amino acid sequence can easily be isolated, changed, and re-inserted. Examples of functional regions of the amino acid sequence are transmembrane spanning regions, immunodominant regions, regions with antibody cross reacting domains, fusion domains and other regions important for immunogenicity and expression such as variable region 1 (V1), variable region 2 (V2), variable region 3 (V3), variable region 4 (V4) and variable region 5 (V5).

It is important to select the restriction enzymes sites with care. By changing the second nucleotide sequence to insert restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence, the third nucleotide sequence must still code for the same amino acid sequence as the second and first nucleotide sequence do. Thus, if necessary, the second nucleotide sequence is redesigned by changing from optimised codons to less optimal codons. It is understood, that the restriction enzyme sites around the nucleotide sequence encoding functional regions of the amino acid sequence should preferably be placed in the terminal region of the nucleotide sequence encoding functional regions of the amino acid sequence. That is preferably outside the nucleotide sequence encoding functional regions of the amino acid sequence, such as 90 nucleotides away, e.g. 81, 72, 63, 54, 45, 36, 27, 21, 18, 15, 12, 9, 6,3 nucleotides away, but could also be inside the nucleotide sequence encoding functional regions of the amino acid sequence, such as 54, 45, 36, 27, 21, 18, 15, 12, 9, 6, 3 nucleotides inside the nucleotide sequence encoding the functional region of the amino acid sequence.

The type of restriction enzyme sites allowed is determined by the choice of expression vector. In certain cases, the number of restriction enzyme sites is limited and it is hard, if not impossible, to place unique restriction enzyme sites around all the nucleotide sequences coding for functional regions of the amino acid sequence. This problem can be solved by dividing the entire nucleotide sequence into pieces, so that each piece comprises only unique restriction enzyme sites. Modifications to each of the piece is performed separately prior to assembly of the pieces. It is preferred that the nucleotide sequence is divided into 9 pieces. In another aspect, the nuclotide sequence is divided into 8 pieces, or 7, or 6, or 5, or 4, or 3, or 2 pieces. It is especially preferred that the nucleotide sequence is divided into 3 pieces.

Thus, the redesign of the second nucleotide sequence is an interaction between the choice of cloning vector, expression vector, selection of restriction enzyme sites, dividing into pieces, and exchange of codons to insert restriction enzyme sites. In a preferred embodiment of the present invention the cloning vector is Bluescript allowing the restriction enzyme sites chosen from the group consisting of: *Eag*I, *Mlu*I, *Eco*RV, *Pst*I, *Cla*I, *Eco*RI, *Xba*I, *Sac*I, *Spe*I, *Xho*I, *Hind*III, *Sac*II, *Not*I, *Bam*HI, *Sma*I, *Sal*I, *Dra*I, *Kpn*I. If other cloning vectors are chosen, other restriction enzyme sites will be available as known by the person skilled in the art.

As a part of the redesigning of the second nucleotide sequence, selected restriction enzyme sites may be removed. The selected restriction enzyme sites to be removed are those sites that are sites of the same type as the ones already chosen above and that are placed within the same piece. The removal of these restriction enzyme sites is performed by changing from optimised codons to less optimal codons, maintaining codons for the same amino acid sequence.

The third nucleotide sequence is redesigned so that the terminal snuts contain convenient restriction enzyme sites for cloning into an expression vehicle. The expression "vehicle" means any nucleotide molecule e.g. a DNA molecule, derived e.g. from a plasmid, bacteriophage, or mammalian or insect virus, into which fragments of nucleic acid may be inserted or cloned. An expression vehicle will contain one or more unique restriction enzyme sites and may be capable of autonomous replication in a defined host or vehicle organism such that the cloned sequence is produced. The expression vehicle is an autonomous element capable of directing the synthesis of a protein. Examples of expression vehicles are mammalian plasmids and viruses, tag containing vectors and viral vectors such as adenovirus, vaccinia ankara, adenoassociated virus, cannarypox virus, simliki forest virus (sfv), Modified Vaccinia Virus Ankara (MVA), and simbis virus. In one embodiment of the invention, the expression vector contains tag sequences. In another embodiment of the invention a bacteria is transformed with an expression plasmid vector and the bacteria is then delivered to the patient. Preferred expression vehicles are simliki forest virus (sfv), adenovirus and Modified Vaccinia Virus Ankara (MVA).

The snuts are produced by techniques well known by the person skilled in the art. The preferred method for synthesising snuts, is herein referred to as "the minigene approach" wherein complementary nucleotide strands are synthesised with specific overhanging sequences for annealing and subsequent ligation into a vector. This can be performed with

two sets of complementary nucleotide strands, or with three sets of complementary nucleotide strands. The minigene approach minimises the known PCR errors of mismatches and/or deletions, which may occur due to hairpins in a GC rich gene with mammalian highly expressed codons. In figures 10-21, the production of a representative selection of snuts is illustrated.

For the production of long snuts, that is snuts of more than about 240 nucleotides, the technique of overlapping PCR is preferred as illustrated in figure 8. Herein two nucleotide strands about 130 nucleotides long with an overlap are filled to obtain a double strand, which is subsequently amplified by PCR.

For the production of multiple snuts with a length of less than about 210 nucleotides, one preferred technique is normal PCR. In a preferred production technique the snuts are synthesised with the same 5' flanking sequences and with the same 3' flanking sequences, as illustrated in figure 9. The advantages of this approach is, that the same PCR primer set can be used for amplification of several different snuts.

As known by the person skilled in the art, special conditions have to be used for each individual PCR reaction and it should be optimised to avoid inherent problems like deletions mismatches when amplificating GC rich genes from synthetic ssDNA material. Whichever of the above mentioned techniques are used, it is well known by the person skilled in the art, that it will be necessary to correct unavoidable mismatches produced either due to the nucleotide strand synthesis material and/or the PCR reaction. This can be performed by site directed mutagenesis techniques.

After the various snuts have been produced, they are assembled into pieces and subsequently into the complete gene. Methods for assembly (such as ligation) are well known by the person skilled in the art.

In a preferred embodiment of the present invention the HIV gene encodes the entire HIV envelope. It is understood that the HIV envelope can be the full length envelope gp160 as well as shorter versions such as gp150, gp140, and gp120 with or without parts of gp41.

As will be known by the person skilled in the art, the HIV is divided into several groups. These groups presently include group M, group O, and group N. Further, the HIV is divided

into subtypes A, B, C, D, E, F, G, H, I, and J. In the present invention subtype B is preferred due to the high prevalence of this subtype in the Western countries.

The determination of groups and subtypes is based on the degree of nucleotide sequence identity in the envelope gene is presently defined as follows: If the sequence identity is more than 90% the viruses belong to the same subtype; If the sequence identity is between 80% and 90% the viruses belong to the same group. If the sequence identity is less than 80% the viruses are considered as belonging to different groups.

One aspect of the invention relates to a nucleotide sequence construct in isolated form which has a nucleotide sequence with the general formula (I), (II), (III), or (IV)

(I) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265gp120}$

(II) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp140}

(III) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp150}

(IV) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp160}- $S_{2060SacII}$- P_5

wherein P_1 designates the nucleotide sequence SEQ ID NO:41, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein $S_{495ClaI}$ designates the nucleotide sequence SEQ ID NO: 7, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

wherein $S_{650\text{-}720EcoRI}$ designates the nucleotide sequence SEQ ID NO: 9, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

wherein P_2 designates the nucleotide sequence SEQ ID NO: 43, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein $S_{1265gp120}$ designates the nucleotide sequence SEQ ID NO: 19, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 70% thereto;

wherein $S_{1265XhoI}$ designates the nucleotide sequence SEQ ID NO: 17, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 80% thereto;

wherein $S_{1465PstI}$ designates the nucleotide sequence SEQ ID NO: 23, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein P_{4gp140} designates the nucleotide sequence SEQ ID NO: 57, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein P_{4gp150} designates the nucleotide sequence SEQ ID NO: 55, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein P_{4gp160} designates the nucleotide sequence SEQ ID NO: 53, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein $S_{2060SacII}$ designates the nucleotide sequence SEQ ID NO: 33, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 98% thereto; and

wherein P_5 designates the nucleotide sequence SEQ ID NO: 59, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto.

The design of the parent synthetic BX08 gp160 envelope cassette gene with its variant length genes gp150, gp140, gp120 is outlined in figure 2.

The nucleotide sequence construct with the formula (I)

(I) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265gp120}$

(visualised in figure 3) (SEQ ID NO: 73) codes for the amino acid sequence of gp120 (SEQ ID NO: 74). This amino acid sequence is the part of the HIV envelope that is secreted. Thus, it contains the immunogenic epitopes without being bound to the cell membrane. This is of particular advantage if the nucleotide sequence construct is used for production of recombinant antigens or for a DNA vaccine as the antibody immune response may be higher to secreted versus membrane bound HIV antigens.

The nucleotide sequence construct with the formula (II)

(II) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp140}

(visualised in figure 4) (SEQ ID NO: 67) codes for the amino acid sequence of gp140 (SEQ ID NO: 68). This amino acid sequence encodes the gp120 and the extracellular part of the gp41 protein. The amino acid sequence is secreted due to the lack of the transmembrane spanning region. This is of particular advantage if the nucleotide sequence construct is used for production of recombinant antigens as the immunogenic and/or antigenic epitopes in the extracellular part of gp41 are included and is of particular advantage for a DNA vaccine as

the antibody immune response may be higher to secreted gp120 versus membrane bound HIV antigens.

The nucleotide sequence construct with the formula (III)

(III) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp150}

(visualised in figure 5) (SEQ ID NO: 69) codes for the amino acid sequence of gp150 (SEQ ID NO: 70). This amino acid sequence contains all of the envelope protein gp160 except the c-terminal tyrosin containing internalisation signals in the intracellular part of gp41. The membrane bound surface expression of the amino acid sequence is thereby maintained and enhanced. Mimicking the organisation of the native epitope conformation may by expected, making this nucleotide sequence construct of particular advantage if the nucleotide sequence construct is used as a vaccine.

The nucleotide sequence construct with the formula (IV)

(IV) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp160}- $S_{2060SacII}$- P_5

(visualised in figure 6) (SEQ ID NO: 71) codes for the amino acid sequence of gp160 (SEQ ID NO: 72) i.e. the entire envelope.

The nucleotide sequence construct designated P_1 comprises the nucleotide sequence encoding the amino acid sequence in the first variable region (V1) and the amino acid sequence in the second variable region (V2). In one embodiment of the invention the first variable region is surrounded by EcoRV and PstI restriction enzyme sites, and the second variable region is surrounded by PstI and ClaI restriction enzyme sites but as stated above, the choice of restriction enzyme sites can alter.

The nucleotide sequence construct designated $S_{650\text{-}720EcoRI}$ comprises the nucleotide sequence encoding the amino acid sequence in the third variable region (V3). In one embodiment of the present invention $S_{650\text{-}720EcoRI}$ is characterised by the restriction enzyme sites EcoRI and XbaI in the terminals.

The nucleotide sequence construct designated P_2 comprises the nucleotide sequence encoding the amino acid sequence of the fourth variable and constant region (V4 and C4). In one embodiment of the present invention the forth variable region is surrounded by *Sac*I and *Xho*I restriction enzyme sites.

The nucleotide sequence construct designated $S_{1265gp120}$ comprises the nucleotide sequence encoding amino acid sequence of the fifth variable and constant region (V5 and C5). $S_{1265gp120}$ further comprises a nucleotide sequence encoding a C-terminal stop codon.

The nucleotide sequence construct designated P_{4gp140} comprises the nucleotide sequence encoding amino acid sequence of the transmembrane spanning region. P_{4gp140} further comprises a nucleotide sequence encoding a C-terminal stop codon prior to the transmembrane spanning region.

The nucleotide sequence construct designated P_{4gp160} comprises the nucleotide sequence encoding amino acid sequence of the transmembrane spanning region (trans membrane spanning domain: TMD). In a preferred embodiment of the present invention the transmembrane spanning region is surrounded by HindIII and SacII restriction enzyme sites.

The term "sequence identity" indicates the degree of identity between two amino acid sequences or between two nucleotide sequences calculated by the Wilbur-Lipman alignment method (Wilbur et al, 1983).

The nucleotide sequence constructs with the formula (I), (II), (III), or (IV) illustrates the flexibility in the present invention. By producing a gene with the described method enables the production of a plethora of antigens with various immunogenic epitopes and various advantages for production and vaccine purposes. To further illustrate the flexibility of the invention, other changes and mutations are suggested below.

In order to improve the immunogenicity of the nucleotide sequence constructs of the invention it is suggested to change the nucleotide sequence such that one or more glycosylation sites are removed in the amino acid sequence. By removal of shielding glycosylations, epitopes are revealed to the immunesystem of the mammal rendering the construct more immunogenic. The increased immunogenicity can be determined by an improved virus neutralisation. Changes in the nucleotide sequence such that one or more N-linked glycosylation sites are removed in the amino acid sequence is well known by the person skilled in the art. Potential glycosylation sites are N in the amino acid sequences N-X-T or N-X-S (wherein X is any amino acid besides P). The glycosylation site can be removed by changing N to any amino acid, changing X to a P, or changing T to any amino acid. It is preferred that N is changed to Q by an A to C mutation at the first nucleotide in the codon, and a C to G mutation at the third nucleotide in the codon. This is preferred to increase the

GC content in the nucleotide sequence construct. As an alternative N is changed to Q by an A to C mutation at the first nucleotide in the codon, and a C to A mutation at the third nucleotide in the codon. Preferred mutations in the synthetic BX08 envelope gene to remove potential N-linked glycosylation sites in V1 and/or V2 are A307C + C309A and/or A325C + C327G and/or A340C + C342A and/or A385C + C387A and/or A469C + C471A. Examples of such changes is illustrated in SEQ ID NOs: 47, 49, and 51.

For historical reasons the HIVs have been divided into syncytia inducing strains and non syncytia inducing strains. The assay to determine whether a strain is syncytia inducing is described in Verrier et al 1997, hereby incorporated by reference. It is presently known, that viruses utilising the CXCR4 co-receptor are syncytia inducing strains. It is also, at the present, known that the binding site for the CXCR4 involves the third variable region (V3). In a preferred embodiment the nucleotide sequence construct is changed to create a binding site for the CXCR4 co-receptor. It is presently performed in the third variable regions, preferably by the mutation G865C + A866G.

It is well established that the HIV envelope comprises immunodominant epitopes. An immunodominant epitope is an epitope that most antibodies from the mammal are directed against. The antibodies directed against these immunodominant epitopes may have little effect in elimination of the virus. It is therefore anticipated that modification of the immunodominant epitopes will induce antibodies directed against other parts of the envelope leading to a better elimination and neutralisation of the virus. By modification is understood any change in the nucleotide sequence encoding an immunodominant epitope in the amino acid sequence such that said amino acid sequence no longer contains an immunodominant epitope. Thus, modification includes removal of the immunodominant epitope and decrease of immunogenicity performed by mutagenesis. In a preferred embodiment of the present invention an immunodominant epitope in the third variable region (V3) is modified, such as deleted or altered. In a much preferred embodiment the nucleotides 793-897 are deleted. In yet another preferred embodiment of the present invention an immunodominant epitope has been removed from gp41, such as deleted. This is performed in P_7 or P_8 by elimination of the nucleotides 1654-1710.

It is anticipated that when gp120 is dissociated from gp41 in a vaccine or antigen, two immunodominant epitopes, one on each protein, are exposed and antibodies are directed against these in the mammal. In the infectious virus, gp120 is coiled on top of gp41 and the gp120/gp41 is most likely organised in a trimer, so that these immunodominant epitopes are

hidden and therefore less elimination of virus is observed. By removing the cleavage site between gp41 and gp120 a full length gp160, gp150, or gp140 can be obtained with a covalent binding between gp41 and gp120. Removal of the cleavage site between gp41 and gp120 is preferably performed by a mutation at position C1423A. An example of such a mutation is illustrated in the mutation of $S_{1265XhoI}$ (SEQ ID NO: 17) to $S_{1265gp160}$ (SEQ ID NO: 21).

In order to stabilise the full length gp160, gp150, and gp140 for example when the cleavage site between gp41 and gp120 has been removed as described above, cysteins can be inserted, preferably inside the gp41 helix creating disulphide bounds to stabilise a trimer of gp41s. In a preferred embodiment of the present invention the cysteins are inserted by the mutation 1618:CTCCAGGC:1625 to 1618:TGCTGCGG:1625. An example of such a change is illustrated in SEQ ID NO: 25.

The above mentioned decrease in immunodominant epitopes combined with the increase in immunogenicity of the other epitopes is expected to greatly enhance the efficacy of the nucleotide sequence construct as a vaccine.

During the production of the nucleotide sequence construct, it is convenient to ligate the snuts into pieces. The pieces, as described above, are characterised by their reversible assembly as there are no duplicate restriction enzyme sites. In a preferred embodiment one piece (herein designated P_3) contains P_1, $S_{495ClaI}$,$S_{650\text{-}720EcoRI}$, and P_2. Another piece (herein designated P_8) contains $S_{1265XhoI}$, $S_{1465PstI}$, and P_{4gp160}. Yet another piece (herein designated P_7) contains $S_{1265XhoI}$, $S_{1465PstI}$, P_{4gp160}, $S_{2060SacII}$, and P_5.

One advantage of the present nucleotide sequence construct is the easy access to exchange and alterations in the content and function of the nucleotide sequence and the encoded amino acid sequence. In one embodiment the nucleotide sequence coding for a functional region or parts thereof of the amino acid sequence is repeated. The repeat could be back-to-back or a functional region or parts thereof could be repeated somewhere else in the sequence. Repeated could mean two (one repetition) but could also be three, six, or nine repeats. In a much preferred embodiment the repetition nucleotide sequence codes for amino acids in the third variable region.

In order to improve the protective capabilities of the invention against infections with HIV, one embodiment of the invention relates to the combination of epitopes. The present

nucleotide sequence construct allows insertion of one or more new nucleotide sequences isolated from another group and/or subtype of HIV and/or isolated from another patient. Hereby a vaccine or antigen with two or more epitopes from two or more HIVs is obtained. In a preferred embodiment, the V3 is replaced by the new nucleotide sequence. In a much preferred embodiment, the new nucleotide sequence codes for amino acids in the third variable region of a different HIV isolate.

In order to improve the efficacy of the vaccine, aiming at raising cellular immunity, a nucleotide sequence coding for a T-helper cell epitope is included in the nucleotide sequence construct. The nucleotide sequence coding for a T-helper cell epitope or a T-helper cell epitope containing amino acid sequence can be put in anywhere in the nucleotide sequence construct as long as it does not interact with the function of the envelope molecule. However, it is preferably placed in the tail of the nucleotide sequence construct or between the leader sequence and the envelope gene. The T-helper epitopes are preferably selected from core proteins such as P24gag or from a non-HIV pathogen such as virus, bacteria, e.g. BCG antigen 85. For a therapeutic vaccine an HIV helper epitope is preferred since the patient is already primed by the HIV infection. For a prophylactic vaccine, a T-helper cell epitope from a frequently occurring non HIV pathogen such as Hepatitis B, BCG, CMV, EBV is preferred. Also, since the synthetic BX08 envelope genes may contain T-helper cell epitopes in addition to important antibody epitopes, the synthetic BX08 vaccine genes can be mixed with other DNA vaccines to improve the efficacy of the other DNA vaccine.

One aspect of the present invention relates to individualised immunotherapy, wherein the virus from a newly diagnosed patient is directly cloned, the envelope or subunits corresponding to snuts or pieces is produced with highly expressed codons, inserted into any of the nucleotide sequence constructs described above and administered to the patient as a vaccine. Hereby a therapeutic DNA vaccine is obtained, that will help the patient to break immunetolerance or induce/reinduce an appropriate immune response. In one embodiment the variable regions of the virus are produced with highly expressed codons and exchanged into any of the nucleotide sequence constructs described above.

In one embodiment of the invention, the nucleotide sequence construct as described above satisfies at least one of the following criteria:

a) serum extracted from a Macaque primate which has been immunised by administration of an expression vector containing the nucleotide sequence construct is capable of eliminating SHIV as determined by quantitative PCR and/or virus culturing.

b) serum extracted from a primate which has been immunised by administration of an expression vector containing the nucleotide sequence construct is capable of neutralising HIV-1 BX08 and /or other HIV-1 strains *in vitro*.

c) serum, extracted from a mouse which has been immunised by administration of an expression vector containing the nucleotide sequence construct four times in intervals of three weeks and boosted after 15 weeks, is capable of decreasing the concentration of HIV-antigen in a culture of HIV, serum or PBMCs by at least 50%. An example of such procudure is shown in example 9.

In one embodiment of the invention, the nucleotide sequence construct of the invention, is used in medicine. That is, it is used as a vaccine, for the production of a recombinant protein, such that the recombinant protein is used as a vaccine, or the nucleotide sequence construct or the recombinant protein is used in a diagnostic composition.

Thus, the nucleotide sequence construct of the invention can be used for the manufacture of a vaccine for the prophylactics of infection with HIV in humans.

Intramuscular inoculation of nucleotide constructs, i.e. DNA plasmids encoding proteins have been shown to result in the generation of the encoded protein in situ in muscle cells and dendritic cells. By using cDNA plasmids encoding viral proteins, both antibody and CTL responses were generated, providing homologous and heterologous protection against subsequent challenge with either the homologous or cross-strain reaction, respectively. The standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA therapeutics of this invention. While standard techniques of molecular biology are therefore sufficient for the production of the products of this invention, the specific constructs disclosed herein provide novel therapeutics which can produce cross-strain protection, a result heretofore unattainable with standard inactivated whole virus or subunit protein vaccines.

The amount of expressible DNA to be introduced to a vaccine recipient will depend on the strength of the transcription and translation promoters used in the DNA construct, and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of about 10 μg to 300 μg is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, inoculation by gene gun preferably DNA coated gold particles, and other modes of administration such as intraperitoneal, intravenous, peroral, topic, vaginal, rectal, intranasal or by inhalation delivery are also contemplated. It is also contemplated that booster

vaccinations are to be provided. It is further contemplated that booster vaccinations with recombinant antigens are to be provided, administered as described above.

The DNA may be naked, that is, unassociated with any proteins, adjuvants or other agents which impact on the recipients immune system. In this case, it is desirable for the DNA to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the DNA may be associated with surfactants, liposomes, such as lecithin liposomes or other liposomes, such as ISCOMs, known in the art, as a DNA-liposome mixture, (see for example WO93/24640) or the DNA may be associated with and adjuvant known in the art to boost immune responses, such as a protein or other carrier. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, detergents, viral proteins and other transfection facilitating agents may also be used to advantage. These agents are generally referred to as transfection facilitating agents and as pharmaceutically acceptable carriers.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used. A wide range of suitable mammalian cells are available from a wide range of sources (e.g. the American Type Culture Collection, Rockland, Dm; also, see e.g. Ausubel et al. 1992). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described e.g. in Ausubel et al 1992; expression vehicles may be chosen from those provided e.g. in P.H. Pouwels et al. 1985.

In one embodiment of the present invention the protein encoded by the nucleotide sequence construct is produced by introduction into a suitable mammalian cell to create a stably-transfected mammalian cell line capable of producing the recombinant protein. A number of vectors suitable for stable transfection of mammalian cells are available to the public e.g. in *Cloning Vectors*: A Laboratory manual (P.H. Pouwels et al. 1985); methods for constructing such cell lines are also publicly available, e.g. in Ausubel et al. 1992.

Standard reference works describing the general principles of recombinant DNA technology include Watson, J.D. et al 1987; Darnell, J.E. et al 1986; Old, R.W. et al, 1981; Maniatis,T. et al 1989; and Ausubel et al.1992.

Figure legends

The invention is further illustrated in the following non-limiting examples and the drawing wherein

Figure 1 provides the codon preference of highly expressed proteins in human cells.

Figure 2 illustrates the outline of gp120, gp140, gp150, and gp160 encoding synthetic genes derived from the wild type sequence at the top. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown. The approximate position of the three restriction enzyme sites dividing the full-length gp160 gene into the three pieces each containing only unique restriction enzyme sites are shown in bold.

Figure 3 building of the synthetic gp120 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown.

Figure 4 building of the synthetic gp140 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown.

Figure 5 building of the synthetic gp150 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown.

Figure 6 building of the synthetic gp160 gene. Variable (V) and constant (C) regions are shown together with the leader peptide (LP) and the transmembrane spanning domain (TMD). The approximate nucleotide positions of the restriction enzyme sites are shown.

Figure 7 illustrates the codons coding amino acids in general

Figure 8 illustrates how overlapping PCR is performed.

Figure 9 illustrates how PCR using conserved flanking ends is performed.

Figure 10 illustrates how $S_{1265XhoI}$ is produced using complementary strands (minigene-approach) technology. The $S_{1265XhoI}$ is ligated from three sets of complementary strands into the vector pBluescript KS^+ between restriction enzyme sites *Xho*I and *Pst*I.

Figure 11 illustrates how $S_{1465PstI}$ is produced. The same approach, as the approach used for the production of $S_{1265XhoI}$, was used except that only two sets of complementary strands were used.

Figure 12 illustrates the assembly of P_1. The $S_{O\text{-}N\text{-}Lang}$ and $S_{235EcoRV}$ are ligated into the *Xba*I and *Pst*I site of the $S_{375PstI}$ containing plasmid.

Figure 13 illustrates the assembly of P_2. The $S_{900XbaI}$ was excerted by *Hind*III and *Sac*I from its plasmid and ligated with $S_{990SacI}$ (*Sac*I-*Spe*I) into the $S_{110SpaI}$ plasmid that was opened at the *Hind*III and *Spe*I sites.

Figure 14 illustrates the assembly of P_3. $S_{495ClaI}$ (*Cla*I-*Eco*RI) and $S_{650\text{-}720EcoRI}$ (*Eco*RI-*Xba*I) and P_2 (*Xba*I-*Xho*I) were ligated simultaneously into the P_1 plasmid opened at the *Cla*I and *Xho*I sites to obtain the P_3 plasmid.

Figure 15 illustrates the assembly of P_{4gp160}. $S_{1890HindIII}$ (*Sac*I-*Hind*III) and $S_{1700eagI}$ (*Hind*III-*Eag*I) were ligated simultaneously into the $S_{1630XhaI}$ plasmid opened by *Sac*II and *Eag*I.

Figure 16 illustrates the assembly of P_5. $S_{2190ClaI}$ (*Cla*I-*Pst*I) and $S_{2330PstI}$ (*Pst*I-*Eco*RI) were ligated into the S_{2425Es} plasmid opened by *Cla*I and *Eco*RI.

Figure 17 illustrates the assembly of P_{8gp160}. $S_{1465PstI}$ (*Xba*I-*Pst*I) and $S_{1265XhoI}$ (*Pst*I-*Xho*I) were ligated into the P_{4gp160} plasmid opened by *Xba*I and *Xho*I.

Figure 18 illustrates the assembly of P_{8gp150}. $S_{1465PstI}$ (*Xba*I-*Pst*I) and $S_{1265XhoI}$ (*Pst*I-*Xho*I) were ligated into the plasmid containing P_{4gp150} with the stop codon. P_{4gp150} plasmid was opened at the *Xba*I and *Xho*I sites for the ligation.

Figure 19 illustrates the assembly of P_{8gp140}. $S_{1465PstI}$ (*Xba*I-*Pst*I) and $S_{1265XhoI}$ (*Pst*I-*Xho*I) were ligated into the plasmid containing P_{4gp140} with a stop codon. P_{4gp140} plasmid was opened at the *Xba*I and *Xho*I sites for the ligation.

Figure 20 illustrates the assembly of P_{8gp41}. Two complementary nucleotide strands 1265gp41S and 1265gp41AS designed with overhang creating a 5' *Xho*I and a 3' *Pst*I restriction enzyme site were anealed and ligated into the piece 8 which is already opened at the *Xho*I and *Pst*I sites whereby S_{1265} is deleted.

Figure 21 illustrates the assembly of P_7. P_8 (*Xho*I-*Sac*II) and $S_{2060SacII}$ (*Sac*II-*Cla*I) were ligated into P_5 plasmid opened at *Xho*I and *Cla*I.

Figure 22a SDS PAGE of ^{35}S-labelled HIV-1 BX08 envelope glycoproteins radio-immuno precipitated from transiently transfected 293 cells using the indicated plasmids. Cell pellet (membrane bound antigens) or cell supernatant (secreted antigens) were precipitated by a polyclonal anti-HIV-1 antibody pool. Lane 1: untransfected cells. Lane 2: supernatant from syn.gp120$_{MN}$ transfected cells. Lane 3: cell pellet from wt.gp160$_{BX08}$ transfected cells. Lane 4: cell pellet from cells co-transfected by wt.gp160$_{BX08}$ and pRev. Lane 5: Mwt. marker. Lane 6: cell pellet from syn.gp160$_{BX08}$ transfected 293 cells. Lane 7: cell pellet from syn.gp150$_{BX08}$ transfected 293 cells. Lane 8: supernatant from syn.gp140$_{BX08}$ transfected cells. Lane 9: supernatant from syn.gp120$_{BX08}$ transfected cells.

Figure 22b is an SDS-PAGE of ^{35}S-labeled HIV-1 BX08 envelope glycoproteins radio-immune precipitated from transiently transfected 293 cells as cell pellet (membrane bound) or cell supernatant (secreted antigens) by anti-HIV-1 antibody pool using the indicated plasmids. Lane 1: untransfected 293 cells. Lane 2: cell pellet from syn.gp160MN transfected 293 cells as positive control (Vinner et al 1999). Lane 3: Cell supernatant from syn.gp120MN transfected 293 cells as positive control (Vinner et al 1999). Lane 4: Cell supernatant from syn.gp120BX08 transfected 293 cells demonstrating a glycoprotein band of 120 kDa. Lane 5: Cell supernatant from syn.gp140BX08 transfected 293 cells demonstrating a glycoprotein band of 120 kDa. Lane 6: Mwt. marker. Lane7 at two different exposure times: Cell pellet from syn.gp150BX08 transfected 293 cells demonstrating a glycoprotein band of 120 kDa (lower gp30 band is not well seen in this exposure). Lane 8: Cell supernatant from syn.gp150BX08 transfected 293 cells showing no secreted proteins (all protein is membrane bound, see lane 7).

Figure 22c show fluorescent microscopy of U87.CD4.CCR5 cells transfected with BX08 gp160 genes plus pGFP. Panel A: cells transfected with empty WRG7079 vector plus pGFP showing no syncytia. Panel B: cells transfected with wild type BX08gp160 gene

plus pGFP showing some syncytia. Panel C: cells transfected with synBX08gp160 plus pGFP showing extreeme degree of syncytia formation. This demonstrates expression, functionality, and tropism of the expressed BX08 glycoprotein with much more expressed functionally active gp160 from the synthetic BX08 gene.

Figure 23 shows the anti-Env-V3 BX08 antibody titers (IgG1). Panels show individual mice DNA immunized with syn.gp140BX08 plasmid either i.m. (left panel) or by gene gun (right panel), respectively. Immunization time points are indicated by arrows.

Figure 24 shows a Western Blotting of (from left to right) one control strip, followed by sere (1:50) from 2 mice i.m. immunized with synBX08gp120, 2 mice i.m. immunized with synBX08gp140, 2 mice i.m. immunized with synBX08gp150, and 2 mice immunized with synBX08gp160, followed by 2 mice gene gun immunized with synBX08gp120, 2 mice gene gun immunized with synBX08gp140, 2 mice gene gun immunized with syn BX08gp150, and 2 mice gene gun immunized with synBX08gp160 respectively. Strip 5 is a mouse 5.1 DNA immunized i.m. with synBX08gp140 plasmid (same mouse as in figure 23). Plasma was examined at week 18. The positing of gp160 (spiked with four coupled gp51), gp120 and gp41 is indicated at the right. A positive reaction to HIV glycoproteins futher demonstrates the mouse anti-HIV immunoglobulin reacting to HIV of a strain (IIIB) different from BX08 to illustrate cross-strain reactivity.

Figure 25 Theoretical example of calculation of the 50% inhibitory concentration (IC_{50}) values. IC_{50} for each mouse serum is determined by interpolation from the plots of percent inhibition versus the dilution of serum.

Figure 26 CTL responses were measured at week 18 to the mouse H-2D^d restricted BX08 V3 CTL epitope (IGPGGRAFYTT) for BALB/c mice (H-2D^d) i.m. immunized at week 0, 9, and 15 with the synthetic vaccine genes: syn.gp120$_{BX08}$, syn.gp140$_{BX08}$, syn.gp150$_{BX08}$, and syn.gp160$_{BX08}$, respectively, and median values of different E:T ratios for groups of mice are shown (26A). Intramuscular DNA immunization with syn.gp150$_{BX08}$ induced a higher CTL reponse when injected i.m. in high amounts versus gene gun inoculation of skin (26B).

Figure 27 Summary of western immuno blotting assay of mice sera (1:40) collected at week 0, 9, and 18 from mice genetically immunized with syn.gp120$_{BX08}$, syn.gp140$_{BX08}$,

syn.gp150$_{BX08}$, syn.gp160$_{BX08}$, wt.gp160$_{BX08}$, and wt.gp160$_{BX08}$ plus pRev, respectively. Percent responders in groups of 17-25 mice against gp120 and gp41 are shown.

Figure 28 IgG anti-rgp120 (IIIB) antibody titers of individual mice inoculated at week 0, 9, 15 (28A), or gene gun immunized at week 3, 6, 9, and 15 (28B) with the syn.gp150$_{BX08}$ DNA vaccine.

Figure 29 IgG antibody titers to HIV-1 rgp120$_{IIIB}$. Median titers are shown from groups of mice i.m. inoculated at week 0, 9, and 15 (29A), or gene gun immunized at week 0, 3, 6, 9, and 15 (29B) with the synthetic genes syn.gp120$_{BX08}$, syn.gp140$_{BX08}$, syn.gp150$_{BX08}$, and syn.gp160$_{BX08}$, respectively.

Examples

Example 1: Designing the nucleotide sequence construct

Initially the overall layout of the nucleotide sequence construct is decided. The overall layout comprises the various derivatives the gene will be expressed as. For BX08 these include, but are not restricted to gp160, gp150, gp140, gp120, and gp41.

Next, the vehicle of expression (plasmid or virus) is to be determined: Preparation for a suitable vector determines both need for leader sequence, terminal restriction enzyme sites and whether or not an N- or C-terminal protein tag is to be considered (Poly-his, Myc-antibody-epitop, etc.). For BX08 a plasmid expression vehicle was chosen. All native wild type HIV codons are systematically exchanged with the codons most frequently represented in a pool of highly expressed human genes (figure 1). By this exchange the amino acid sequence is conserved while the nucleotide sequence is dramatically altered. Thus, gene structures like overlapping reading frames (e.g. *vpu*, *rev*, and *tat*) or secondary structures (e.g. RRE) are most likely destroyed whereas protein cleavage sites, and glycosylation sites are maintained. The 100% amino acid identity between wtBX08 and synthetic BX08 in the present examples should be calculated after the initial Ala-Ser amino acid sequence, as that sequence is a part of the 6 amino acid sequence long *Nhe*I restriction enzyme site.

Depending on the restriction enzyme sites located in the expression vector it is decided which restriction enzyme sites can be present (tolerated) throughout the finished gene construct. The terminal restriction enzyme sites of the synthetic gene must remain unique to enable cloning into the vector chosen for expression. General requirements for restriction enzyme sites of choice: Preferably creating cohesive ends facilitating ligation, creating no compatible ends with adjacent restriction enzyme sites (e.g. *Bam*HI/*Bgl*II), and being efficient cutters. For BX08 the restriction enzyme sites accepted were the ones present in the polylinker of the pBluescript cloning vectors (*Eag*I, *Mlu*I, *Eco*RV, *Pst*I, *Cla*I, *Eco*RI, *Xba*I, *Sac*I, *Spe*I, *Xho*I, *Hind*III, *Sac*II, *Not*I, *Bam*HI, *Sma*I, *Sal*I, *Dra*I, *Kpn*I with the exception of *Bgl*II and *Nhe*I). This was decided to satisfy the original cloning strategy using individual cloning of snuts in pBluescript with restriction enzyme cleaved (trimmed) ends after PCR amplification, which is not necessary when blunt-end cloning and assembling of complementary oligonucleotides are employed. All locations at which the selected restriction enzyme sites can be introduced by silent mutations (keeping 100% loyal to the amino acid sequence) are identified using the SILMUT software or equivalent.

From these possible restriction enzyme sites, a selection of restriction enzyme sites are introduced by silent nucleotide substitutions around functional regions of choice of the corresponding gene (e.g. RRE) or gene products (e.g. variable region 1 (V1), V2, V3, CD4 binding area, transmembrane domain, and regions of immunological significans, etc.).

Restriction enzyme sites are located at terminal positions of subcloned snuts (building entities) but additional restriction enzyme sites may be present within subunits. For BX08 the construct was initially to be cloned in the WRG7079 vector containing a tPA-leader sequence. Cloning sites were 5'-*Nhe*I → *Bam*HI-3'. The entire humanised BX08 sequence was divided into thirds: 5'-*Nhe*I → *Xho*I → *Sac*II → *Bam*HI-3'. These sites were chosen in this particular order because it resembles the polylinker of pBluescript (KS^-) enabling successive ligations of the assembled thirds in this cloning vector. Within these thirds restriction enzyme sites were kept unique. Next, restriction enzyme sites were placed to flank the functional regions chosen as follows:

A. (5'-V1): *Eco*RV-235: Between C1 and V1. Alternatives: 3×*Hind*III (already excluded because exclusive use at position 1890) or *Eco*RV.

B. (V1-3'): Only alternative *Pst*I 375.

C. (5'-V2): as B.

D. (V2-3'): Alternatives: *Spe*I, *Cla*I 495. *Cla*I chosen because it is closer to V2.

E. *Eco*RI 650 placed because next possible site was too far away.

F. (5'-V3): *Bgl*II 720 was the alternative closest to the V3 region and further more unique.

G. (V3-3'): Alternatives *Xho*I (excluded) and *Xba*I 900 located very close to the V3 loop.

H. (5'-V4) *Sac*I 990: alternatively *Eco*RI or *Bam*HI (both excluded)

I. (V4-3'): Alternatives *Spe*I 1110, *Kpn*I 1145, *Pst*I 1135. *Pst*I already used, *Spe*I chosen because of distance to previous site (*Sac*I 990).

J. (5'-V5): *Xho*I initially determined.

K. (Fusion peptide-3') *Pst*I 1465 was the closest alternative to *Xho*I 1265.

L. (5'-Immunodominant region): *Xba*I 1630 chosen among *Eco*RV (blunt end), *Pst*I and *Xho*I (both already used).

M. (Immunodominant region-3'): *Eag*I 1700 perfect location.

N. (C34 and C43 -3' (Chan, Fass, et al. 1997), and 5'-trans membrane domain): *Sac*II. No alternatives.

O. (Trans membrane domain -3'): *Sac*II 2060 already present.

P. *Cla*I 2190 perfect position in relation to previous RE-site.

Q: PstI perfect position in relation to previous RE-site.

R. *Eco*RI 2400 introduced to facilitate later substitution of terminal snut.

S. *Bam*HI 2454 determined by the WRG7079 vector.

Remove undesired restriction enzyme sites by nucleotide substitutions (keeping loyal to the amino acid sequence). Nucleotide substitution should preferably create codon frequently used in highly expressed human genes (figure 1). If that is not possible, the codons should be the selected from the regular codons (figure 7). The substitutions made to the second nucleotide sequence to obtain desired restriction enzyme sites are shown in Table 2.

Table 2 lists silent nucleotide substitutions in the humanised BX08 envelope sequence. Substitutions were made to create or delete restriction enzyme sites.

Position:	substitution	Remarks:
138	c → g	creates Mlu I site on pos. 134-139
240	c → t	creates EcoRV site on pos. 238-243
501	c → a	creates Cla I site on pos. 501-506
502	a → t	do
503	g → c	do
504	c → g	do
657	c → a	creates EcoRI site on pos. 656-661
660	c → t	do
724	c → a	creates Bgl II site on pos. 724-729
726	c → g	do
727	a → t	do
728	g → c	do
729	c → t	do
840	c → t	EagI site is eliminated
904	a → t	creates Xba I site on pos. 904-909
905	g → c	do
906	c → t	do
907	c → a	do
909	c → a	do
994	a → t	creates Sac I site on pos. 990-995
995	g → c	do
1116	c → t	creates SpeI site on pos. 1114-1119
1119	c → t	do
1273	a → t	creates XhoI site on pos. 1272-1277
1274	g → c	do
1275	c → g	do
1293	c → t	Bgl II site is eliminated
1443	c → t	BstXI site is eliminated
1452	g → c	do
1467	c → t	PstI site on pos. 1466-1471
1470	c → a	do
1590	g → c	PstI site on pos. 1588-1593 is eliminated
1620	g → c	PstI site on pos. 1618-1623 is eliminated
1638	c → t	creates XbaI site on pos. 1638-1643
1641	g → a	do
1653	g → c	PstI site is eliminated
1687	a → t	PstI site is eliminated
1688	g → c	PstI site is eliminated
1710	c → g	creates EagI site on pos. 1709-1714
1758	c → t	Bgl II site is eliminated
1875	g → c	PstI site is eliminated
1893	c → a	Hind III on pos. 1893-1898
1897	c → t	do
1944	c → t	Bgl II site is eliminated

Position:	substitution	Remarks:
2199	c → t	Cla I site on pos. 2198-2203
2202	c → t	do
2203	c → t	do
2253	c → g	SacII site is eliminated
2292	g → c	PstI site is eliminated
2320	a → t	PstI site on pos. 2321-2326 is eliminated
2321	a → t	do
2322	g → t	do
2325	c → a	do
2430	c → a	creates EcoRI site on pos. 2429-2434
2433	c → t	do

Example 2: synthesis of oligos

In order to clone the individual snuts, nucleotide strands were synthesised or purchased. In total 28 synthetic nucleotide strands were synthesised. Nucleotide strands were synthesised by standard 0.2 μmol β-cyanoethyl-phosphoramidite chemistry on an Applied Biosystems DNA synthesiser model 392, employing 2000 Å CPG columns (Cruachem, Glasgow, Scotland), acetonitrile containing less than 0.001% water (Labscan, Dublin, Ireland) and standard DNA-synthesis chemicals from Cruachem, including phosphoramidites at 0.1 M and Tetrahydrofuran/N-methylimidazole as cap B solution. The nucleotide strands O-N-C and 119MS-RC (for cloning of snut O-N-Lang), 650-E-BG and 720-XBAC-31 (for cloning of snut 650-720-EcoRI), 2425esup and 2425ESdo (for cloning of snut 2425-E-S) were synthesised with 5' end "trityl on" and purified on "Oligonucleotide Purification Cartridges" (Perkin Elmer, CA, USA) as described by the manufacturer. Other nucleotide strands (235-ECO5, 375-pst1.seq, 495-Cla1.seq,900-XbaI, 990-sac1, 1110-SPE, 1630-Xba.seq, 1700-Eag.seq, 17-Eag.seq, 1890-Hind.MPD, 2060-sac, 2190-cla, 2330-pst) were synthesised with 5' end "trityl off" and purified by standard ethanol precipitation. Oligoes 1265-1UP, 1265-1DO, 1265-2UP, 1265-2DO, 1265-3UP, 1265-3DO, 1465-1UP, 1465-1DO, 1465-2UP, 1465-2DO were purchased from Pharmacia.

Example 3: Cloning of snuts

The nucleotide sequence construct was designed in 17 DNA small pieces called snuts (Table 3) encompassing important structures like variable and constant regions each flanked with restriction enzyme (RE) sites to facilitate cassette exchange within each third of the gene: *Nhe*I-*Xho*I, *Xho*I-*Sac*II, *Sac*II-*Bam*HI.

Each snut was cloned individually in a commercial vector (pBluescriptKS or pMOSblue) and kept as individual DNA plasmids, named after the snut which gives the nucleotide position of the RE in the BX08.

Table 3 list the snuts by their name and cloning vector.

Name	Cloning vector:
$S_{O\text{-}N\text{-}Lang}$	pMOSblue
$S_{235EcoRV}$	pMOSblue
$S_{375PstI}$	pBluescriptSK
$S_{495ClaI}$	pMOSblue
$S_{650\text{-}720EcoRI}$	pMOSblue
$S_{900XbaI}$	pMOSblue
$S_{990SacI}$	pMOSblue
$S_{1110SpeI}$	pMOSblue
$S_{1265XhoI}$	pBluescriptSK
$S_{1465PstI}$	pBluescriptSK
$S_{1630XbaI}$	pBluescriptSK
$S_{1700EagI}$	pBluescriptSK
$S_{1890HindIII}$	pBluescriptSK
$S_{2060SacII}$	pMOSblue
$S_{2190ClaI}$	pMOSblue
$S_{2330PstI}$	pMOSblue
S_{2425ES}	pBluescriptSK

Three principally different methods were used to obtain the dsDNA corresponding to each of the 17 snuts needed to build the synthetic BX08 genes.

1) "Overlapping" PCR: is based on the use of two ssDNA template nucleotide strands (forward and reverse) that complement each other in their 3'-end (figure 8). During the first PCR cycle, both templates annealed to each other at the 3'-ends allowing the full-length polymerisation of each complementary strand during the elongation step. The newly polymerized dsDNA strand are then amplified during the following cycles using an adequate forward and reverse primers set (figure 8).

Snut O-N-LANG: ($S_{O\text{-}N\text{-}Lang}$) two ng of the forward template nucleotide strands O-N-C and 2 ng of the reward template-nucleotide strand 119MS-RC were mixed together with 50pmoles of the forward primer O-N-LANG-5 (5'-CTAGCTA-GCGCGGCCGACCGCCT -3') and 50pmoles of the reverse primer O-N-LANG-3 (5'-CTCGATATCCTCGTGCATCTGCTC -3') in a 100µl PCR reaction volume containing 0.2mM dNTP's, 1x ExpandHF buffer with $MgCl_2$ (1.5mM) and 2.6 units of enzyme mix (Expand™ High fidelity PCR system from Boehringer Mannheim). The PCR was performed with the PE Amp 9600 thermocycler (Perkin Elmer) using the following cycle conditions: initial denaturation at 94°C for 30 sec., followed by 30 cycles of 94°C for 15 sec., 65°C for 30 sec., 72°C for 45 sec., with a final elongation at 72°C for 5 min., and cooling to 4°C.

Snut 650-720-EcoRI: ($S_{650\text{-}720EcoRI}$) PCR amplification was performed as described for snut O-N-LANG. One μg of the forward ssDNA template-oligonucleotide 650-E-BG and 1 μg of the reverse ssDNA template-oligonucleotide 720-XBAC were mixed with 40pmoles of the forward primer 650-E-5 (5'-CCGGAATT-CGCCCCGTGGTGAGCA-3') and 40 pmoles of the reverse primer 720-X-3 (5'-CTGCTCTAGAGATGTTGCAGTGGGCCT-3').

2) "Normal" PCR amplification: Eleven nucleotide strands: 235-ECO5, 375-pst1, 900-xba1, 990-sac1, 1110-SPE, 1630-XBA, 1700-EAG, 1890-HIN, 2060-sac, 2190-cla, and 2330-pst, were designed with common 5' and 3' flanking sequences which allowed PCR amplification with the same primer set (Forward primer : BX08-5 (5'-AGCGGATAACAATTTCACACAGGA-3') and revers primer : BX08-3 (5'-CGCCAGGGTTTTCCCAGTCACGAC-3') (Figure 9). The 495-Cla1 oligonucleotide was designed without a common flanking sequence and was therefore amplified with a specific set of primers 495-5N/495-3N (5'-GAATCGAT-CATCACCCAG-3' and 5'-GACGAATTCCGTGGGTGCACT-3'). Each oligonucleotide was resuspended in 1ml of water and kept as a stock solution (approximatively 0.2 mM). PCR amplification was performed with the Expand™ High Fidelity PCR System from Boehringer Mannheim (Cat. No. 1759078). Four concentrations of template nucleotide strand were systematically used: undiluted stock solution, stock solution 10^{-1}, stock solution 10^{-2}, stock solution 10^{-3}. One to 5 μl of synthetic ssDNA template was amplified using the following conditions: BX08-5 (0.5μM), BX08-3 (0.5μM), 4 dNTP's (0.2mM), 1x ExpandHF buffer with $MgCl_2$ (1.5mM) and 2.6 units of enzyme mix. The PCR was performed using the PE Amp 9600 thermocycler (Perkin Elmer) using the following cycle conditions: initial denaturation at 94°C for 15 sec., followed by 30 cycles of 94°C for 15 sec., 65°C for 30 sec. and 72°C for 45 sec., with a final elongation at 72°C for 7 min., and cooling to 4°C.

3) Minigene approach : This method was used to synthesise $S_{1265XhoI}$, $S_{1465XbaI}$ and S_{2425ES}.
Snut 2425-E-S (S_{2425ES}): 100 picomoles of each oligonucleotide 2425ES-up (35-mer ; 5'-AATTCGCCAGGGCTTCGAGCGCGCCCTGCTGTAAG-3') and 2425ES-do (35-mer ; GATCCTTACAGCAGGGCGCGCTCGAAGC-CCTGGCG-3') were mixed together in a 100 μl final volume of annealing buffer containing NaCl 25mM , Tris 10mM and 1mM EDTA. After denaturation at 94°C for 15 min., the mixed oligonucleotides were allowed to anneal at 65°C during 15min.. The annealing temparature was allowed to slowly decrease from the 65°C to room temperature (22°C) during overnight incubation. The resulting double-strand dsDNA fragments harbored EcoRI- and BamHI-restriction sites overhangs that allowed direct cloning in pBluescript KS(+) vector using standard cloning techniques (Maniatis 1996).

Snut 1265-XhoI ($S_{1265XhoI}$): This snut was built according to the strategy depicted in figure 10. Three minigenes were constructed following the same method described for snut 2425-E-S. These minigenes are named 1265-1, 1265-2 and 1265-3. The minigene 1265-1 results from the annealing of the oligonucleotides 1265-1up (68-mer ; 5'-TCG AGC AGC GGC AAG GAG ATT TTC CGC CCC GGC GGC GGC GAC ATGC GCG ACA ACT GGC GCA GCG AGC T-3') and 1265-1do (68-mer ; 5'-GTA CAG CTC GCT GCG CCA GTT GTC GCG CAT GTC GCC GCC GCC GGG GCG G AAA ATC TCC TTG CCG CTG C-3'). 1265-2 results from the annealing of 1265-2up (61-mer ; 5'-GTA CAA GTA CAA GGT GGT GAA GAT CGA GCC CCT GGG CAT CGC CCC CAC CAA GGC CAA GCG C-3') and 1265-2do (63-mer ; 5'-CAC GCG GCG CTT GGC CTT GGT GGG GGC GAT GCC CAG GGG CTC GAT CTT CAC CAC CTT GTA CTT-3'). Finally, 1265-3 results from the annealing of 1265-3up (69-mer ; 5'-CGC GTG GTG CAG CGC GAG AAG CGC GCC GTG GGC ATC GGC GCT ATG TTC CTC GGC TTC CTG GGC GCT GCA-3') and 1265-3do (59-mer ; 5'-GCG CCC AGG AAG CCG AGG AAC ATA GCG CCG ATG CCC ACG GCG CGC TTC TCG CGC TGC AC-3'). Each minigene were designed in order to present single strand overhangs at their 5' and 3'- ends that allow easy ligation and XhoI-PstI direct cloning into pBlueScript KS+ vector.

Snut 1465-PstI ($S_{1465PstI}$): Two minigenes were constructed following the same methode described for snut 2425-E-S. These minigenes are named 1465-1 and 1465-2. The minigene 1465-1 was obtained after annealing of 1465-1up (90-mer : 5'-GGC AGC ACC ATG GGC GCC GCC AGC CTG ACC CTG ACC GTG CAG GCC CGC CAG CTG CTG AGC GGC ATC GTG CAG CAG CAG AAC AAC CTG CTG-3') and 1465-1do (98-mer : 5'-CGC GCA GCA GGT TGT TCT GCT GCT GCA CGA TGC CGC TCA GCA GCT GGC GGG CCT GCA CGG TCA GGG TCA GGC TGG CGG CGC CCA TGG TGC TGC CTG CA-3'), whereas minigene 1465-2 results from the annealing of 1465-2up (78-mer ; 5'-CGC GCC ATC GAG GCC CAG CAG CAC CTG CTC CAG CTGA CCG TGT GGG GCA TCA AGC AGC TCC AGG CCC GCG TGC TGG CT-3') and 1465-2do (78-mer ; 5'-CTA GAG CCA GCA CGC GGG CCT GGA GCT GCT TGA TGC CCC ACA CGG TCA GCT GGA GCA GGT GCT GCT GGG CCT CGA TGG-3'. Each minigene were designed in order to present single strand overhangs at their 5' and 3'- ends that allow easy ligation and PstI-XbaI direct cloning into pBlueScript KS+ vector using standard cloning techniques (Maniatis) (see figure 11).

Example 4: assembly of snuts to pieces.

The snut genes were then assembled into pieces (Table 4) so that unique restriction enzyme sites or mutagenesis can be used within each of these. This strategy will require fewer assemblings for optimal use of the cassette system. The following piece clones were made and kept individually for construction of the synBX08 gp160 gene (Figure 6):

Table 4 lists pieces by their name and their snut composition.

Piece name	snut composition	vector
P_1	$S_{O\text{-}N\text{-}LANG}$-$S_{235EcoRV}$-$S_{375PstI}$	pBluescriptSK
P_2	$S_{900XbaI}$-$S_{990SacI}$-$S_{1110SpeI}$	pMOSblue
P_3	P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2	pBluescriptSK
P_{4gp160}	$S_{1630XbaI}$-$S_{1700EagI}$-$S_{1890HinIII}$	pBluescriptSK
P_5	$S_{2190ClaI}$-$S_{2330PstI}$-S_{2425ES}	pBluescriptKS
P_7	P_{8gp160}-$S_{2060SacII}$-P_5	pBluescriptKS
P_{8gp160}	$S_{1265XhoI}$-$S_{1465PstI}$-P_{4gp160}	pBluescriptKS

Piece 1: The building strategy is shown in figure 12.

Preparation of the insert DNA: Five to 15μg of each plasmid O-N-LANG-cl7 and 235-EcoRV-cl5N, respectively, were double-digested by XbaI/EcoRV, and PstI/EcoRV, according to classical RE digestion procedure (Maniatis). The RE digestion products, were agarose gel purified according classical method (Maniatis). All RE digests were loaded on a 3% Nusieve 3:1(FMC), TBE 0.5X agarose gel and submitted to electrophoresis (7 Volts/mm during 2-3hours) until optimal fragment separation. The agarose-band containing the DNA fragments that correspond to the snut's sequence sizes (243-bp for O-N-LANG and 143-bp for 235-EcoRV) were excised from the gel. The DNA was extracted from agarose by centrifugation 20min at 5000g using a spin-X column (Costar cat#8160). *Preparation of the vector:* The snut 375-Pst1 klon1 was used as plasmid vector. Five μg were digested with XbaI and Pst1. Removal of the polylinker XbaI/PstI fragment was performed by classical agarose gel purification, using a 0.9% Seakem-GTG agarose, TBE 0.5X gel. The linearised plasmid DNA was extracted from the agarose by filtration through spin-X column. All purified DNA fragments were quantified by spectrophotometry. *Ligation:* All three DNA fragments O-N-LANG (XbaI/EcoRV), 235-EcoRV (PstI/EcoRV) and 375-PstI(XbaI/PstI), were ligated together by classical ligation procedure, using an equimolar (vector:insert1:insert2) ratio of 1:1:1. Thus for, 200 ng (0.1 pmole) of XbaI/PstI-linearised 375-PstI-cl1 were mixed with 16 ng of O-N-LANG (XbaI/EcoRV) and 10 ng of 235-EcoRV (PstI/EcoRV) in a final reaction volume of 20μl of 1X ligation buffer containing 10U of T4 DNA ligase (Biolabs, cat#202S). The ligation was allowed overnight at 16°C. *Transformation:* Competent XL1-Blue bacteria (Stratagene cat#200130, transformation efficiency > $5 \bullet 10^6$ col/μg) were transformed by classical heat-chock procedure : 1/10th of the pre-chilled ligation reaction was mixed with 50μl of competent bacteria. The mixture was allowed to stand in ice during 30 min. Bacteria were heat-shocked at 42°C during 45 sec. and then left 2 min. on ice before being resuspended in 450 μl of SOC medium. Transformed bacteria were incubated 1 hour at 37°C under shaking (250rpm) and plated on LB-ampicilin agar plates. The recombinant clones

were allowed to grow 16 hours at 37°C. *Colony screening:* 10 to 50 recombinant colonies were screened by direct PCR screening according to the protocole described into the pMOSBlue blunt-ended cloning kit booklet (RPN 5110, Amersham). Each colony was picked and resuspended in 50µl of water. DNA was freed by a boilling procedure (100°C, 5 min).Ten µl of bacterial lysate were mixed to 1 µl of a 10mM solution of premixed 4 dNTP's , 1 µl of M13reverse primer (5pmoles/µl, 5'-CAGGAAACAGCTATGAC-3'), 1 µl of T7 primer (5pmoles/µl, 5'-TAATACGACTCACTATAGGG-3'), 5µl of 10x Expand HF buffer 2 (Boehringer Mannheim, cat#1759078), 0.5µl of Enzyme mix (Boehringer Mannheim, 5U/µl) in a final volume of 50µl. DNA amplification was performed with a thermo-cycler PE9600 (Perkin-Elmer) using the following cycling parameters: 94°C, 2min, 35 cycles(94°C, 30sec; 50°C, 15sec; 72°C,30sec); 72°C, 5min; 4°C hold. Five µl of the PCR products were analysed after electrophoresis on a 0.9% SeakemGTG , 0.5xTBE agarose gel. *Nucleotide sequence confirmation:* ds-DNA was purified from minicultures of the selected clones with the JETstar mini plasmid purification system (Genomed Inc.). Sequencing was performed using M13reverse and T7 primers and with the Big DyeTM Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer, Norwalk, Connecticut, P/N4303152) and the ABI-377 automated DNA sequenator (Applied Biosystems, Perkin-Elmer,Norwalk, Connecticut). Data were processed with the Sequence Navigator and Autoassembler softwares (Applied Biosystems, Perkin-Elmer,Norwalk, Connecticut).

Piece 2: The strategy for building that piece is depicted in figure 13. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The linearised plasmid 1110-SpeI-cl24M1 was used as vector after being digested by HindIII and SpeI, and agarose gel purified.
- A 166-bp HindIII/SacI, obtained from snut 900-XbaI-cl15, as well as a 130-bp SacI/SpeI fragment, obtained from snut 990-SacI-cl14, were agarose gel purified.
- Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated in an one step ligation.
- 100µl of competent SCS110 bacteria (Stratagene cat# 200247) were transformed with 1/10th of the ligation products according to the manufactor instruction.
- Direct colony PCR screening was performed using T7 primer and pMOS-R (5'-GTTGTAAAACGACGGCCAG-3').

Piece 3: The strategy for building that piece is depicted in figure 14. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid piece1-cl33 was linearised by ClaI and XhoI, in order to be used as vector, and agarose gel purified.
- A 161-bp ClaI/EcoRI fragment, obtained from 495-ClaI-cl135M1 as well as a 254-bp EcoRI/XbaI fragment, obtained from 650-720-EcoRI-cl39, and a 374-bp XbaI/XhoI fragment, obtained from piece2-cl4, were agarose gel purified.
- Equimolar amount (0.1 pmole) of each of these 4 DNA fragments were mixed and ligated together.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using M13Reverse and T7 primers.

Piece 4 gp160: The strategy for building that piece is depicted in figure 15. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid 1630-XbaI-cl2 was linearised by SacII/ EagI digestion and agarose gel purified, in order to be used as vector.
- A 190-bp EagI/HindIII fragment, obtained from snut 1700-EagI-cl4, as well as a 177-bp SacII/HindIII fragment, obtained from snut 1890-HindIII-cl8, were agarose gel purified.
- Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated in an one step ligation.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using M13Reverse and T7 primers.

Piece 4-gp150: PCR-based site-directed mutagenesis was performed on double-stranded plasmid-DNA from piece4-cl4 according an adaptation of the ExSite™ PCR-Based Site-Directed Mutagenesis Kit procedure (Stratagene cat#200502)(Weiner, M.P., Costa, G.L., Schoettlin, W., Cline, J., Marthur, E., and Bauer, J.C. (1994) Gene 151:119-123). The mutations introduced are shown in bold letters in the primer sequences below. PCR amplification was performed with the Expand™ High Fidelity PCR System (Boehringer Mannheim, cat#1759078). Briefly, 1.5 µg, 0.5 µg or 0.1 µg of circular dsDNA was mixed with 1.5 pmoles of P4M2S (5'-TCTGGAAGCTCAGGGGGCTG**CA**T**C**CCTGGC-3') and 1.5

pmoles of P4M2AS (5'-CCCGCCTGCCCGTG**TGA**C**GGATCC**AGCTCC-3') in a final volume of 50µl containing 4 dNTPs (250µM each), 1x Expand HF buffer 2 (Boehringer Mannheim, cat#1759078), 0.75µl of Enzyme mix (Boehringer Mannheim, 5U/µl). The PCR was performed with a PE9600 thermo-cycler (Perkin-Elmer Corporation) under the following cycling parameters : 94°C, 2min ; 15 cycles (94 °C, 45 sec ; 68 °C, 4 min); 72°C, 7min and 4 °C, hold. PCR products were phenol:chloroform extracted and precipitated (Maniatis). Plasmid template was removed from PCR products by DpnI treatment (Biolabs)(Nelson, M., and McClelland, M., 1992) followed by ethanol-precipitation. Amplicons were resuspended in 50 µl steril water, and phosphorylated according the following procedure: 7.5 µl of amplicons were mixed with 0.5µl of 100mM DTT, 1 µl of 10x pk buffer and 1 µl of pk mix enzyme (pMOSBlue blunt-ended cloning kit, Amersham , cat#RPN 5110). DNA kinasing was allowed 5min at 22°C. After heat-inactivation (10min, 75°C) of the pk enzyme, 1µl of ligase (4units, Amersham , cat#RPN 5110) was directly added to the pk reaction. The ligation was allowed overnight at 22°C. 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation reaction according to the classical protocol (Maniatis). Insertion of mutations was checked by sequencing.

Piece 4-gp140: PCR-based site-directed mutagenesis was performed on piece 4-cl4, according to the procedure described for piece4-gp150 except that the primers P4M1AS (5'-TGTG**TG**ACTG**ATTGAGG**ATC**C**CCAACTGGC-3') and P4S (5'-AGCTTGCCCACTTGTCCAGCTGGAGCAGGT-3') were used.

Snut 1265-XhoI-gp120 : PCR-based site-directed mutagenesis was performed on plasmid 1265-XhoI-cl2M1 according to the procedure described for piece4-gp150 except that the primers 1265MAS (5'-CTTCTCGCGCTGCACCACGCGGCGCTTGGC-3') and 1265M2S (5'-CGCGCC**TA**GGGCATCGGCGCTATGTTCCTC-3') were used.

Snut 1265-XhoI-gp160/uncleaved : PCR-based site-directed mutagenesis was performed on plasmid 1265-XhoI-cl2M1 according to the procedure described for piece4-gp150 except that the primers 1265MAS (5'-CTTCTCGCGCTGCACCACGCGGCGCTTGGC-3') and 1265M2S (5'-**A**GCGCCGTGGGCATCGGCGCTATGTTCCTC-3') were used.

Snut 1465-PstI-CCG : PCR-based site-directed mutagenesis was performed on plasmid 1465-PstI-cl25 according to the procedure described for piece4-gp150 except that the primers 1465MAS (5'-CTGCTTGATGCCCCACACGGTCAGCTG-3') and 1465MS (5'-**TGCTGC**GGCCGCGTGCTGGCTCTAGA-3') were used.

Piece 5 : The strategy for building that piece is depicted in figure 16. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid 2425-ES-cl2 was linearised by ClaI/EcoRI digestion and agarose gel purified, in order to be used as vector.
- A 129-bp PstI/ClaI fragment, obtained 2190-ClaI-cl6M15, as well as a 114-bp PstI/EcorI fragment, obtained from 2330-PstI-cl8, were agarose gel purified.
- Equimolar amount (0.1 pmoles) of the three DNA fragments described above were ligated in an one step ligation.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using T3 (5'-ATTAACCCTCACTAAAG-3') and T7 primers.

piece 8 : The strategy for building that piece is depicted in figure 17. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:

- The plasmid piece4-cl4 was linearised by XbaI/XhoI and agarose gel purified, in order to be used as vector.
- A 200-bp XhoI/PstI fragment, obtained from 1265-XhoI-cl2M1 as well as a 178-bp PstI/XbaI fragment, obtained from 1465-PstI-cl25 were agarose gel purified.
- Equimolar amount (0.1 pmole) of these 3 DNA fragments were mixed and ligated together.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using T3 and T7 primers.

piece 8-gp150 : The strategy for building that piece was identical to that of piece 8, except that piece4-cl4M3 was used as vector (figure 18).

piece8-gp150/ uncleaved : The strategy for building that piece is identical to that of piece 8, except that piece4 gp160-cl4M3 is used as vector and a 200-bp XhoI/PstI fragment, obtained from snut 1265-XhoI-gp160/uncleaved as well as a 178-bp PstI/XbaI fragment, obtained from snut 1465-PstI-CCG are used like inserts.

piece 8-gp140 : The strategy for building that piece was identical to that of piece 8, except that piece4-cl4M5 was used as vector (figure19).

piece8-gp140/ uncleaved : The strategy for building that piece is identical to that of piece 8, except that piece4-cl4M5 is used as vector and a 200-bp XhoI/PstI fragment, obtained from snut 1265-XhoI-gp160/uncleaved as well as a 178-bp PstI/XbaI fragment, obtained from snut 1465-PstI-CCG are used like inserts.

Piece8-gp41 : The strategy for building that piece is depicted in figure 20. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 63 bp linker is to be made according to the method described for snut 2425-ES, the minigene appraoch. Thus for 2 complementary oligonucleotides: 1265-gp41S(5'-**TCGAG**gctagcGCCGTGGGCATCGGCGCTATGTTCCTCGGCTTCCTGGGCG**ctgca**-3') and 1265-gp41AS (5'-gCGCCCAGGAAGCCGAGGAAC-ATAGCGCCGATGCCCACGGCgctagcC-3' should be annealed together. This synthetic linker will be directly ligated into the *XhoI /PstI* sites of piece8-klon13 from which the snut 1265-XhoI-cl 2M1 would have been removed.

piece7 : The strategy for building that piece is depicted in figure 21. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described above for piece1, except the following:
- The plasmid piece5-cl1 was linearised by ClaI/XhoI and agarose gel purified, in order to be used as vector,.
- A 798-bp XhoI/SacII fragment, obtained from piece8-cl13 as well as a 140-bp SacII/ClaI fragment, obtained from 2060-SacII-cl21 were agarose gel purified.
- The ligation of the 3 fragments was performed using a vector:insert ratio of 1:1, 1:2 or 1:5.
- 50µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation products according to the protocole described for piece1.
- Direct colony PCR screening was performed using M13Reverse and T7 primers.

Example 5: assembly of genes

synBX08 gp160 gene : The strategy for building that gene is depicted in figure 6. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for piece1.
20 µg of the expression plasmid WRG7079 were digested by NheI/BamHI. Plasmid DNA-

ends were dephosphorylated by Calf Intestin Phosphatase treatment (CIP, Biolabs) (Maniatis) to avoid autoligation of any partially digested vector. CIP enzyme was heat-inactivated and removed by classical phenol-chloroforme extraction. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a *1194*-bp XhoI/BamHI fragment, obtained from piece7-cl1, were agarose gel purified. The ligation was performed using a vector:insert ratio of 1:1 or 1:2. Fifty µl of competent XL1Blue bacteria were transformed with 1/10th of the ligation product according to the protocole described for piece1. After transformation bacteria were plated on LB-kanamycin agar plates. Direct PCR colony screening was performed using the primer set WRG-F (5'-AGACATAATAGCTGACAGAC-3') and WRG-R (5'-GATTGTATTTCTGTCCCTCAC-3'). The nucleotide sequence was determined according the methods described above for piece1.

synBX08 gp150 gene : The strategy for building that gene is depicted in figure 5. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a 800-bp XhoI/BamHI fragment, obtained from piece 8-gp150-cl26, were agarose gel purified and then ligated into the NheI/BamHI WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.
For construction of the synthetic BX08 gp150, piece4 was mutated to Piece4gp150 whereby a tyrosine -> cysteine was changed and a stop codon was introduced after the transmembrane spanning domaine (*TMD*), followed by a BamHI cloning site. A new piece8gp150 was constructed composed of snut1265/snut1465/piece4gp150.

synBX08 gp150/uncleaved gene : RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies are performed according the same procedures described for synBX08 gp160 gene. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a 800-bp XhoI/BamHI fragment, obtained from piece 8-gp150/uncleaved, are agarose gel purified and then are ligated into the NheI/BamHI WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.

synBX08 gp 140 gene : The strategy for building that gene is depicted in figure 4. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a 647-bp XhoI/BamHI fragment, obtained from piece 8-gp140-cl2, were agarose gel purified

and then ligated into the NheI/BamHI sites of WRG7079. The ligation was performed using a vector:insert ratio of 1:1 or 1:2. For construction of the synthetic BX08 gp140, piece4 was mutated to Piece4gp140 whereby a stop codon was introduced just before the *TMD* followed by a BamHI cloning site. A new piece8gp140 was constructed composed of snut1265/1465/piece4gp140.

synBX08 gp140/uncleaved gene : RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies are performed according the same procedures described for synBX08 gp160 gene. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a 800-bp XhoI/BamHI fragment, obtained from piece 8-gp140/uncleaved, are agarose gel purified and then ligated into the NheI/BamHI WRG7079 sites. The ligation was performed using a vector:insert ratio of 1:1 or 1:2.

synBX08 gp 120 gene : The strategy for building that piece is depicted in figure 3. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. A 1277-bp NheI/XhoI fragment, obtained from piece3-cl27, as well as a 206-bp XhoI/BamHI fragment, obtained from 1265-XhoI-gp120-clM5, were agarose gel purified and then ligated into the NheI/BamHI sites of WRG7079. The ligation was performed using a vector:insert ratio of 1:1 or 1:2. For construction of the synthetic BX08 gp120, snut 1265 was mutated to $S_{1265gp120}$ to introduce a stop codon at the gp120/gp41 cleavage site followed by a BamH1 cloning site.

The gp160, gp150, gp140, and gp120 genes are cloned (NheI-BamHI) and maintained in an eucaryotic expression vectors containing a CMV promotor and a tPA leader, but other expression vectors may be chosen based on other criteria e.g. antibiotic resistance selection, other leader sequences like CD5 etc, presence or not of immune stimulatory sequences etc.

SynBX08 gp41 gene : The strategy for building that gene is depicted in figure 20. RE digestion, DNA fragments purification, ligation as well as direct PCR screening of recombinant colonies were performed according the same procedures described for synBX08 gp160 gene. Piece 8-gp41 is ligated with snut 2060-SacII-klon21 and piece 5 as already decribed for the construction of piece 7, creating piece 7-gp41 (P_{7gp41}). Subsequently the piece 7-gp41 containing the entire gp41 gene will be cloned in WRG7079 using the NheI and BamHI sites.

Example 6a: High expression by codon optimization

To analyse the expression of glycoproteins from the wild type and synthetic BX08 envelope genes RIPA was performed on transfected mammalian cell lines. Both cell membrane associated and secreted HIV-1 glycoproteins from the cell supernatants were assayed. The envelope plasmids were transfected into the human embryonic kidney cell line 293 (ATCC, Rockville, MD) or the mouse P815 (H-2D^d) cell line using calcium phosphate (CellPhect Transfection kit, Pharmacia). For radio immune precipitation assay (RIPA), transfected cells were incubated overnight, washed twice and incubated for 1 hour with DMEM lacking cysteine and methionine (Gibco). Then the medium was replaced with medium containing 50 µCi per ml of [^{35}S] cysteine and 50 µCi/ml of [^{35}S] methionine (Amersham Int., Amersham, UK) and incubation continued overnight. Cells were centrifuged, washed twice with HBSS and lysed in 1 ml ice-cold RIPA buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 50 mM EDTA, 1% Nonidet P-40, 0.5% sodiumdeoxycholate) to detect *membrane bound* Env glycoproteins. The cell lysates were centrifuged for 15 min. at 100,000 × g to remove any undissolved particles and 100 µl immune precipitated with protein A-sepharose coupled human polyclonal IgG anti-HIV antibodies (Nielsen et al., 1987). For analysis of secreted Env glycoproteins 500 µl of the 5 ml supernatants from transfected cells were incubated with protein A-sepharose coupled human polyclonal IgG anti-HIV antibodies. After washing three times in cold RIPA buffer and once in PBS, the immuno precipitates were boiled for 4 min. in 0.05 M Tris-HCl, pH 6.8, 2% SDS, 10% 2-mercaptoethanol, 10% sucrose, 0.01% bromophenol blue and subjected to SDS PAGE (Vinner et al., 1999). Electrophoresis was carried out at 80 mV for 1 hour in the stacking gel containing 10% acrylamide, and at 30 mV for 18 hours in the separating gradient gel containing 5-15% acrylamide. Gels were fixed in 30% ethanol-10% acetic acid for 1 hour, soaked for 30 min. in En3Hance (Dupont #NEF 981), washed 2 ×15 min. in distilled water, dried and autoradiography performed on Kodak XAR-5 film. Transfection of human 293 cells with the syn.gp120$_{BX08}$ and syn.gp140$_{BX08}$ genes, respectively, resulted in high amounts of only secreted HIV-1 glycoproteins (Fig. 22a, lane 9 and 8). Thus, the synthetic gene in the absence of *rev* expresses the HIV-1 surface glycoprotein of the expected size which is recognised by human anti-HIV-1 antisera. The expression of BX08 gp120 was Rev independent and with roughly the same high amount of gp120 from the syn.gp120MN gene (Fig. 22a, lane 2). Fig. 22a, lane 6 and lane 7 shows the expression of only membrane bound gp160 and gp150 from 293 cells transfected with syn.gp160$_{BX08}$ and syn.gp150$_{BX08}$ plasmids, respectively. Also transfection with wt.gp160$_{BX08}$ plasmid resulted in a significant expression of membrane bound gp160 despite the absence of Rev (Fig. 22a, lane 3). Co-transfection with equimolar amounts of Rev encoding plasmid seemed to increase this expression somewhat (Fig. 22a, lane 4). This is seen despite the

lower transfection effectivity using two plasmids and the use of only half the amount of wt.gp160$_{BX08}$ DNA when combined with pRev. The amounts of secreted HIV-1 glycoproteins from gp120 and gp141 accumulating in the cell supernatants seemed higher than the amounts of cell associated glycoproteins at the time of harvesting of the cells. Interestingly, the amounts of gp160 produced from the "humanized" gene were about equal to the amounts produced by the wt.gp160$_{BX08}$ + pRev genes, respectively (Fig. 22a, lane 4 and 6). The processing of gp160, gp150 and gp140 into gp120 plus a gp41, or fractions of gp41, produced from wild type or synthetic genes in the 293 cell-line did not function well under these experimental conditions. Same phenomenon was seen in RIPA from 293-CD4 cells and HeLa-CD4 cells infected by HIV-1$_{MN}$ (Vinner et al., 19999). Because of the absence of CCR5 these cell-lines could, however, not be infected by HIV-1 strain BX08.

Example 6b: Radio immuno precipitation assay (RIPA) of synthetic BX08 transfected cells showing expression of glycoproteins from synthetic BX08 env plasmids

The synthetic envelope plasmid DNA were transfected into the human embryonic kidney cell line 293 (ATCC, Rockville, MD) using calcium phosphate (CellPhect Transfection kit, Pharmacia). For immune precipitation analysis, transfected 293 cells were treated and analysed according to the method described in example 6a.

To analyse expression from these genes, an SDS-PAGE of the ^{35}S-labelled HIV-1 envelopes, immune precipitated from the transfected cells is shown in figure 22b. Both cell-membrane associated and secreted HIV-1 envelope glycoproteins in the cell supernatants were assayed. Transfection of 293 cells with the synthetic BX08 gene encoding gp120 (syn.gp120BX08) in lane 4, and syn.gp140BX08 (lane 5) that did not contain *rev* encoding regions, resulted in abundant amounts of HIV-1 gp120. Thus, the expressions were Rev independent and expressed in roughly same high amounts as the syn.gp120MN and syn.gp160MN genes (lane 3 and 2, respectively) already showed by our group and others to be markedly increased in comparisson with HIV MN wild type genes including *rev* (Vinner et al 1999).

Transfection with syn.gp150 plasmid (lanes 7 and 8) resulted in significant expression of membrane associated gp120 and low detactable amounts of truncated form of gp41 (cell pellet in lane 7) with no detectable HIV-1 glycoprotein in the cell supernatant lane 8. It is concluded that the synthetic BX08 genes expres the envelope glycoproteins of expected size which are recognised by human anti-HIV-1 antiserum.

Exampel 6C FACS

To quantitate the surface expression of HIV glycoproteins from the wild type and synthetic BX08 envelope genes transfection experiments were done and cell surface expression examined by FACS (flow cytometer).

10 µg of the BX08 envelope plasmid (wild type BX08gp160 or synBX08gp160) plus 10 µg of an irrelevant carrier plasmid pBluescript were used to transfect a 80-90% confluent layer of 293 cells in tissue culture wells (25 cm^2) using the CellPect kit (Pharmacia). After 48 hours cells were Versene treated, washed and incubated with a mouse monoclonal IgG antibodies to HIV gp120 (NEA-9301, NEN™, Life-Science Products Inc., Boston) for time 30 min. on wet ice followed by washing in PBS, 3% FCS and incubation with Phyto-Erytrin (PE) labelled rat anti-mouse IgG1 (Cat #346270, Becton Dickinson) according to the manufacture. After washing the cells were fixed in PBS, 1% paraformaldehyd, 3% FCS, and analysed on a FACS (FACScan, Becton-Dicknsson). Table 5 show in duplicate expression of BX08 gp160 from 11 % of the cells transfected with wild type BX08 (number 1 and 2) compared to the 48 % of cells expression BX08 glycoprotein when transfected with the synthetic gene (number 3 and 4). Thus, a several fold higher expression is obtained using the synthetic BX08 gene.

Table 5 FACS analysis of 293 cells transfected with synBX08gp160 (No 1 and 2) and wt.gp16+BX08 (No3 and 4) and stained with monoclonal antibodies to surface expressed HIV glycoproteins. A higher expression was obtained with the synthetic gene (mean 48%) as compared to the wild type gene (mean 11 %).

	50 ul	45 ul	A	B	C	C - A	C - B
1	syn.gp160BX08 +	pBluescript SK+	2,57	2,85	36,91	34,34	34,06
2	syn.gp160BX08 +	pBluescript SK+	2,83	2,14	58,42	55,59	56,28
3	wt.gp160BX08 +	pBluescript SK+	1,95	1,52	7,51	5,56	5,99
4	wt.gp160BX08 +	pBluescript SK+	2,97	1,42	14,41	11,44	12,99

A: No primary antibody added (control for unspecific secondary Ab binding)
B: Neither Primary Ab nor Secondary Ab added (autoflouroscense control)
C: Primary Ab and secondary Ab added.

Example 6D Analyses of the surface expression and biological functionality

To analyse the surface expression and biological functionality from the wild type and synthetic BX08 envelope genes transfection experiments were done and cell fusion microscopically studied using HIV envelope receptor expressing cells.

10 μg of the BX08 envelope plasmid (wt.BX08gp160 or syn.BX08gp160 or empty WRG7079 vector plasmid) plus 5 μg of a plasmid (pEGFP, Clonetech) expressing green fluorescent protein (GFP) were transfected into 2 x 10^6 adherent U87.CD4.CCR5 cells (NIH AIDS Res. & Reference program, catalog #4035) stabely expressing CD4 and CCR5, using the CellPhect transfection kit (Pharmacia). After 48 hours the cells were examined by microscopy and photographed (Fig 22c).

Fig 22c panel A show the negative control (empty WRG7079 plus pGFP) giving no syncytia. Panel B show cells transfected with the wild type BX08 gp160 plasmid where cell-to-cell fusion (syncytia) is seen. Panel C show cells transfected with the same amounts of synBX08gp160 plasmid and demonstrating a much higher degree of cell-cell fusion. In fact most or all of the cells in the culture plate were fused at this time. This experiment show surface expression of functional HIV gp160 with tropism to the CCR5 receptor, as well as a much higher expression and biological activity from the synthetic BX08 gene as compared to the wild type equivalent.

Example 7: Gene inoculation of mice for immunization

6-7 weeks old female BALB/c mice were purchased from Bomholdtgaard, Denmak. Microbiological status was conventional and the mice were maintained in groups of 4/5 per cage with food and water ad libitum and artificially lighted 12 hours per day. Acclimatization period was 2 days. Mice were anaesthetized with 0.2 ml i.p. of rohypnol:stesolid (1:3, v/v) and DNA inoculated by either i.m. injection of 50 μl 2 mg/ml of plasmid DNA in each tibia anterior muscle at week 0, 9, and 15 and terminated week 18; or gene gun inoculated on shaved abdominal skin using plasmid coated gold particles (0.95 μm particles, 2 μg DNA/mg gold, 0.5 mg gold/shot, 50-71% coating efficiency) with the hand held Helios® gene gun device (BioRad) employing compressed (400 psi) Helium as the particle motive force. Mice were gene gun vaccinated at week 0, 3, 6, 9, 15, and terminated week 18.

Example 8: Serological assays

Western blotting. The induction of a humoral response to gp120 and gp41 antigens by *in vivo* expression of the encoded glycoproteins from the synthetic BX08 genes was examined by western immuno blotting (Figure 27). Mouse antisera (1:40) were evaluated in western blotting using the commercial HIV BLOT 2.2 strips (Genelabs Diagnostic). The conjugate was a 1:200 dilution of the alkaline phosphatase-conjugated rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark). Buffers, incubation condition and colour development were

used according to the manufacturer. In these western blotting strips the gp160 band from HIV-1 IIIB contain of an oligomeric form of gp41 in a higher concentration than the monomeric gp41 band on the strip (Genelabs Diagnostic). HIV-1_{IIIB} lysate is used in these commercial strips where the gp160 band is composed by addition of tetrameric gp41.All preimmune sera tested negative in western blotting. Mice inoculated with syn.$gp120_{BX08}$ showed antibody response to the heterologous gp120 of HIV-1 IIIB. Inclusion of the extracellular part of gp41 in the gene syn.$gp140_{BX08}$ induced antibody reaction to both gp120 and gp41 in all mice. This confirms the *in vivo* expression of BX08 gp120 and the extracellular part of gp41. DNA vaccination with syn.$gp160_{BX08}$ encoding the membrane bound glycoprotein induced antibodies to gp120 and gp41 in 50% and 64% of the mice, respectively. DNA vaccination with syn.$gp150_{BX08}$ induced detectable antibodies to gp120 and gp41 in 41% and 53%, respectively. Induction of different levels of antibodies could explain the difference in numbers of positive reactive mice sera in this qualitative western blotting.

ELISA. Mouse anti HIV-1 gp120 antibodies were measured by indirect ELISA. Briefly, wells of polystyrene plates Maxisorb (Nunc) were coated for 2 days at room temperature with HIV-1 IIIB recombinant gp120 (Intracel) at 0.2 μg/100 μl of carbonate buffer, pH 9.6. Before use the plates were blocked 1 hour at room temperature with 150 μl/well of washing buffer (PBS, 0.5 M NaCl, 1% Triton-X-100) plus 2% BSA and 2% skimmilk powder. After 3 x 1 min. washings, mouse plasma was added at 100 μl/well diluted in blocking buffer and ELISA plates incubated for 90 min. at room temperature using a microtiter plate shaker. As standard curve we used a mouse monoclonal antibody to a conserved part of gp120 between V5-C5 (MRDNWRSELYKY) (#NEA-9301, NEN™ Life Science Products, Inc., Boston, MA). As calibration control included on each plate we used a plasma pool from 10 mice vaccinated with BX08 gp120. Plates were again washed 5 x 1 min. and incubated 1 hour at room temperature with 100 μl/well of HRP-conjugated rabbit anti-mouse IgG (#P260, Dakopatts, Glostrup, Denmark) diluted 1:1000 in blocking buffer. Colour was developed with 100 μl/well of peroxidase enzyme substrate consisting of 4 mg of *o*-phenylenediamine in 11 ml water plus 4 μl hydrogen peroxide (30%, w/w). The enzyme reaction was terminated after 30 min. by 150 μl/well of 1M H_2SO_4. The optical density (OD) of wells was measured at 492 nm using a microplate photometer (Molecular Devices, Biotech-Line, Denmark). Anti-HIV-gp120 IgG titers were expressed as the reciprocal plasma dilution resulting in an OD_{492nm} value of 0.500. Mouse anti-HIV-1 BX08 antibodies were also measured by indirect peptide ELISAs as described above using a BX08 V3 peptide (SIHIGPGRAFYTTGD) (Schafer, Copenhagen, Denmark).

The IgG antibody response to HIV-1_{IIIB} rgp120 quantitated by ELISA is seen in Fig. 28 and Fig. 29. No background activity was observed in preimmune sera or in sera from 4 mice immunized with empty WRG7079 vector in parallel with the BX08 genes. All mice inoculated with the synthetic BX08 genes either by gene gun or by i.m. injection responded and showed a persistent and high titered (about 100-10,000) IgG response to rgp120 as exemplified in Fig. 4. When comparing the median titers for groups of mice (Fig. 29) a moderate antibody response was observed with the wt.$gp160_{BX08}$. Intramuscular and gene gun immunization with a mixture of wt.$gp160_{BX08}$ plasmid plus Rev encoding plasmid did not increase this antibody response. This was found even when both plasmids were coated onto the same gold particles to ensure co-transfection of single target cells. However, to ensure inoculation of equal amounts of total DNA only half of the amount of wt.BX08 plasmid was used when mixing with pRev which may have contributed to the lower antibody response when pRev was included. A 5-fold improvement of the antibody response was obtained using the syn.$gp160_{BX08}$ gene. This antibody response seemed further improved using the syn.$gp150_{BX08}$ gene where the cytoplasmic internalization signals were eliminated but only using gene gun inoculation. For both the gene gun inoculation of skin and i.m injection the highest antibody titers to rgp120 were induced by genes encoding *secreted* gp120/gp140 glycoproteins versus *membrane bound* gp150/gp160 glycoproteins, respectively. In general, equal antibody and ELISA titers to rgp120 were obtained using gene gun and i.m. injection of the BX08 vaccine genes.

Example 9: Neutralization assay

Mouse plasma was diluted in culture medium (RPMI-1640 medium (Gibco) supplemented with antibiotics (Gibco), Nystatin (Gibco) and 10% FCS (Bodinco)) and heat inactivated at 60°C for 30 min. Of the HIV-1 strain BX08 (50 $TCID_{50}$ per ml propagated in PBMC) 250 µl was incubated for 1 hour at room temperature with 250 µl dilution of mouse serum (four five-fold dilutions of mouse serum, final dilutions 1:20 to 1:2500). After incubation 1×10^6 PBMC in 500 µl culture medium was added to the virus-serum mixture and incubated overnight at 37°C in 5% CO_2. Subsequently, eight replicates of 10^5 PBMC in 200 µl culture medium were cultured in 96-well culture plates (Nunc) at 37°C in 5% CO_2. After seven days in culture the concentration of HIV antigen in the culture supernatant was quantitated using HIV antigen detection ELISA (Nielsen et al., 1987).

This ELISA is performed using human IgG, purified from high titered patient sera, both as capture antibody and biotin-linked as detecting antibody. In brief, anti-HIV-capture IgG diluted 1:4000 in PBS, 100 µl/well, are coated onto Immunoplates (Nunc) overnight at 4°C. After washing five times in washing buffer 100 µl of supernatants are applied and incubated overnight

at 4°C. Plates are washed 5 times before incubation with 100 µl HIV-IgG conjugated with biotin diluted 1:1000 in dilution buffer, plus 10% HIV-1 sero-negative human plasma for 3 hours at room temperature. Five times 1 min. washing in washing buffer are followed by 30 min. incubation with 100 µl of 1:1000 avidine-peroxidase (Dako P347 diluted in dilution buffer). Six times 1 min. washings, 5 in washing buffer and the last one are done in dH_2O before colour is developed with 100 µl of peroxidase enzyme substrate consisting of 4 mg of OPD in 11 ml water plus 4 µl hydrogen peroxide (30 %, w/w). The enzyme reaction is terminated after 30 minutes by additional 150 µl of 1M H_2SO_4.

The HIV antigen concentration in cultures, preincubated with mouse serum, was expressed relatively to cultures without mouse serum (culture medium), and the percentage inhibitions of the different dilutions of mouse serum were calculated. The 50% inhibitory concentration (IC_{50}) for each mouse serum was determined by interpolation from the plots of percent inhibition versus the dilution of serum, and the neutralizing titer of the serum was expressed as the reciprocal value of the IC_{50}. In each set-up a human serum pool known to neutralise other HIV-1 strains was included in the same dilutions as the mouse serum as a calibratin control. For assay of neutralization of the heterologous SHIV89.6P the MT-2-cell-killing format was used (Crawford et al., 1999). The assay stock of SHIV89.6P was grown in human PBMC.

The neutralizing IC_{50} antibody titers of plasma pools from 10 mice from each group were measured at different time points (week 0, 9, and 18). A positive background in some preimmune sera and thus in all week 0 serum pools was noted even after dilution and heat inactivation that was found earlier to lower this background. In general the neutralizing titers to BX08 virus of such serum pools were transient and low ranging from 1:6-1:150 above background (data not shown). A possible cross-neutralization reaction to a heterologous, primary HIV-1 envelope was tested using the SHIV89.6P which is relevant in macaque models of AIDS and serum pools from mice DNA immunized i.m. with syn.$gp140_{BX08}$. Preimmune serum had a titer of 1:37, which is indicative of a slightly positive background, whereas the 18 week p.i. serum had a positive neutralizing titer of 1:254 above background.

Example 10: CTL assay

The cellular immune response in mice following gene gun or i.m. genetic immunization with the different vaccine plasmids were examined (Fig. 26). Spleen was removed aseptically and gently homogenised to single cell suspension, washed 3 times in RPMI-1640 supplemented with 10% FCS and resuspended to a final concentration of 5×10^7 cell/ml. The cells were then incubated 5 days with mitomycin-C treated (50 µg/ml for 1 hour) mouse P815 ($H\text{-}2D^d$)

stimulator cells at a ratio of 10:1 in medium supplemented with 5 x 10^{-5} M β-mercaptoethanol. For assay of CTL response to HIV-1 BX08, P815 stimulator cells and target cells were pulsed with 20 μg/ml of the HIV-1 BX08 V3 peptide containing a conserved murine H-2D^d restricted CTL epitope (IGPGRAFYTT) (Lapham et al., 1996). After stimulation, splenocytes were washed three times with RPMI-1640 supplemented with 10% FCS and resuspended to a final concentration of 5 x 10^6 cells/ml. 100 μl of cell suspension was added in triplicate to U-bottom 96-well microtiter plates and a standard 4 hour ^{51}Cr-release assay performed (Marker et al., 1973).

All synthetic BX08 plasmids induced a high specific CTL response thus confirming the *in vivo* expression and *in vivo* immunogenicity. The highest CTL response was obtained with syn.gp150_{BX08} followed by syn.gp120_{BX08}-syn.gp140_{BX08}, and syn.gp160_{BX08}, respectively. Thus, the CTL response induced did not correlate with the antigen being secreted or not. However, i.m. DNA immunization with syn.gp150_{BX08} containing six putative CpG motifs induced a higher CTL response than gene gun immunization (Fig. 26). This difference could be explained by the high amount of DNA used in the i.m. injections.

The T-lymphocyte cytokine profile of spleen cells after ConA stimulation as well as serum antibody IgG_{2a}/IgG_1 at week 18 were investigated. Neither the IFNγ/IL-4 nor the IgG_{2a}/IgG_1 ratios, which both reflects a Th1-type of immune response, were significantly higher for the i.m. immunized mice when compared with gene gun immunized mice (student t-test and Mann-Withney U-test). Thus, the CTL response did not correlate with a certain Th-type of response and the DNA immunization technique did not bias the immune response using synthetic BX08 genes.

Example 11: Antibody responses to DNA vaccination with synBX08 env plasmid

A relatively low and variable antibody response (1 of 10 mice) was obtained with gene gun inoculation of the syn.gp140BX08 plasmid vaccine starting at week 9, figure 23, right panel. A higher numbers of responders 3/10 with high IgG1 antibody responses at an earlier onset (week 3-9) was obtained with the syn.gp140BX08 plasmid using i.m. injection, left panel. Sera from later time points may show more responders and/or higher titers but are not assayed. However, these results show the induction of an antibody response to the BX08 V3 peptide by DNA vaccination using one of the described synthetic BX08 constructs.

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Claims

1. A method for producing a nucleotide sequence construct comprising the following steps:
a) obtaining a first nucleotide sequence of an HIV gene from a patient within the first 12 months of infection;
b) designing a second nucleotide sequence utilising the most frequent codons from mammalian highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence of a) encodes
c) redesigning the second nucleotide sequence of b) so that restriction enzyme sites surrounds the regions of the nucleotide sequence which encode functional regions of the amino acid sequence and so that selected restriction enzyme sites are removed thereby obtaining a third nucleotide sequence encoding the same amino acid sequence as the first and the second nucleotide sequence of a) and b) encode;
d) redesigning the third nucleotide sequence of c) so that the terminal snuts contain convenient restriction enzyme sites for cloning into an expression vehicle;
e) producing the snuts between restriction enzyme sites of c) and terminal snuts of d);
f) assembling the snut of step e) to form a nucleotide sequence construct.

2. A method according to claim 1, wherein the HIV gene is the gene encoding the envelope.

3. A method according to claim 1 or 2, wherein the HIV gene encodes one or more Gag proteins.

4. A method according to any of the preceding claims, wherein the HIV in step a) is in group M, O or N

5. A method according to claim 4, wherein the HIV is a group M virus.

6. A method according to any of the preceding claims, wherein the HIV is subtype A, B, C, D, E, F, G, H, I, or J.

7. A method according to claim 6, wherein the HIV is subtype B.

8. A method according to any of the preceding claims wherein the first nucleotide sequence is obtained by direct cloning.

9. A method according to any of the preceding claims, wherein the HIV in step a) is isolated with the first 11 months of infection, such a 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0.5 month after infection.

10. A method according to any of the preceding claims, wherein the redesigning in step c) is carried out after the second nucleotide sequence of step b) has been divided into pieces, so that each piece comprises only different restriction enzyme sites.

11. A method according to claim 10, wherein the second nucleotide sequence of step b) is divided into 9 pieces, or 8, or 7, or 6, or 5, or 4, or 3, or 2 pieces.

12. A method according to claim 11, wherein the second nucleotide sequence of step b) is divided into 3 pieces.

13. A method according to any of the preceding claims, wherein the second nucleotide sequence of step b) is designed utilising the most frequent codons from human highly expressed proteins to encode the same amino acid sequence as the first nucleotide sequence of step a) encodes.

14. A nucleotide sequence construct obtainable by the method of any of claims 1-13.

15. A nucleotide sequence construct according to claim 14, wherein the nucleotide sequence encoding the amino acid sequence in the first variable region is surrounded by *Eco*RV and *Pst*I restriction enzyme sites.

16. A nucleotide sequence construct according to claims 14 or 15, wherein the nucleotide sequence encoding the amino acid sequence in the second variable region is surrounded by *Pst*I and *Cla*I restriction enzyme sites.

17. A nucleotide sequence construct according to any of claims 14-16, wherein the nucleotide sequence encoding the amino acid sequence in the third variable region is surrounded by *Cla*I and *Eco*RI restriction enzyme sites.

18. A nucleotide sequence construct according to any of claims 14-17, wherein the nucleotide sequence encoding the amino acid sequence in the transmembrane spanning region is surrounded by *Hind*III and *Sac*II restriction enzyme sites.

19. A nucleotide sequence construct according to any of claims 14-18, wherein the nucleotide sequence encoding the amino acid sequence on both sites of the cleavage site is surrounded by *Pst*I and *Xba*I restriction enzyme sites.

20. A nucleotide sequence construct in isolated form which has a nucleotide sequence with the general formula (I), (II), (III), or (IV) or subsequences thereof

(I) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265gp120}$

(II) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp140}

(III) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp150}

(IV) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp160}- $S_{2060SacII}$- P_5

wherein P_1 designates the nucleotide sequence SEQ ID NO:41, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein $S_{495ClaI}$ designates the nucleotide sequence SEQ ID NO: 7, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95%thereto;

wherein $S_{650\text{-}720EcoRI}$ designates the nucleotide sequence SEQ ID NO: 9, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 95% thereto;

wherein P_2 designates the nucleotide sequence SEQ ID NO: 43, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein $S_{1265gp120}$ designates the nucleotide sequence SEQ ID NO: 19, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 70% thereto;

wherein $S_{1265XhoI}$ designates the nucleotide sequence SEQ ID NO: 17, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 80% thereto;

wherein $S_{1465PstI}$ designates the nucleotide sequence SEQ ID NO: 23, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 90% thereto;

wherein P_{4gp140} designates the nucleotide sequence SEQ ID NO: 57, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein P_{4gp150} designates the nucleotide sequence SEQ ID NO: 55, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein P_{4gp160} designates the nucleotide sequence SEQ ID NO: 53, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto;

wherein $S_{2060SacII}$ designates the nucleotide sequence SEQ ID NO: 33, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 98% thereto; and

wherein P_5 designates the nucleotide sequence SEQ ID NO: 59, a nucleotide sequence complementary thereto, or a nucleotide sequence with a sequence identity of at least 85% thereto.

21. A nucleotide sequence construct according to claim 20, with the formula (I)

(I) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265gp120}$

22. A nucleotide sequence construct according to claim 20, with the formula (II)

(II) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp140}

23. A nucleotide sequence construct according to claim 20, with the formula (III)

(III) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp150}

24. A nucleotide sequence construct according to claim 20, with the formula (IV)

(IV) P_1-$S_{495ClaI}$-$S_{650\text{-}720EcoRI}$-P_2-$S_{1265XhoI}$- $S_{1465PstI}$- P_{4gp160}- $S_{2060SacII}$- P_5

25. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_1.

26. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{495ClaI}$.

27. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{650\text{-}720EcoRI}$.

28. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_2.

29. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{1265gp120}$.

30. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{1265XhoI}$.

31. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{1465PstI}$.

32. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_{4gp140}.

33. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_{4gp150}.

34. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_{4gp160}.

35. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{2060SacII}$

36. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_5.

37. A nucleotide sequence construct with a sequence identity of more than 85% to the nucleotide sequence construct in any of claims 20-35.

38. A nucleotide sequence construct according to claim 37, wherein the sequence identity is more than 90% such as more than 95%, 98%, or 99%.

39. A nucleotide sequence construct according to claim 37, wherein the sequence identity is 100%.

40. A nucleotide sequence construct according to any of claims 14-39, coding for an HIV envelope or parts thereof with an improved immunogenicity obtained by mutating the nucleotide sequence construct of any of claims 14-39 such that one or more glycosylation sites in the amino acid sequence have been removed.

41. A nucleotide sequence construct according to claim 40 with a mutation at positions A307C + C309A and/or A325C + C327G and/or A340C + C342A and/or A385C + C387A and/or A469C + C471A or any combination of those.

42. A nucleotide sequence construct according to any of claims 14-41, coding for an HIV envelope or parts thereof with a binding site for the CXCR4 co-receptor in the third variable region.

43. A nucleotide sequence construct according to claim 42 with a mutation at positions G865C + A866G.

44. A nucleotide sequence construct according to any of claims 14-43, coding for an HIV envelope or parts thereof, wherein an immunodominant epitope has been modified.

45. A nucleotide sequence construct according to claim 44, wherein an immunodominant epitope in the third variable region has been modified.

46. A nucleotide sequence construct according to claim 45 with a deletion of nucleotides 793-897.

47. A nucleotide sequence construct according to claim 44, wherein an immunodominant epitope has been removed from gp41.

48. A nucleotide sequence construct according to any of claims 14-47, coding for an HIV envelope or parts thereof, wherein the cleavage site between gp41 and gp120 is removed.

49. A nucleotide sequence construct according to claim 48 with a mutation at position C1423A.

50. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence P_1, $S_{495ClaI}$, $S_{650\text{-}720EcoRI}$, and P_2.

51. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{1265XhoI}$, $S_{1465PstI}$-, and P_{4gp140}.

52. A nucleotide sequence construct according to claim 20 consisting essentially of the subsequence $S_{1265XhoI}$, $S_{1465PstI}$, P_{4gp160}, $S_{2060SacII}$, and P_5.

53. A nucleotide sequence construct according to any of claims 14-52, further comprising a nucleotide sequence repeat coding for a functional region of the amino acid sequence.

54. A nucleotide sequence construct according to claim 53, wherein the nucleotide sequence repeat codes for amino acids in the third variable region.

55. A nucleotide sequence construct according to any of claim 14-54, further comprising a nucleotide sequence coding for a T-helper cell epitope containing sequence.

56. An expression vehicle selected from a group of viral vectors consisting of simliki forest virus (sfv), adenovirus and Modified Vaccinia Virus Ankara (MVA), further comprising a nucleotide sequence construct according to any of claim 14-55.

57. A method of individualised immunotherapy wherein the virus from a newly diagnosed patient is directly cloned, the envelope is produced with highly expressed codons, inserted into any of the nucleotide sequence constructs of claims 14-55, and administered to the patient.

58. Use of a nucleotide sequence construct according to any of claims 14-55 in medicine.

59. Use of a nucleotide sequence according to any of claims 14-55 for the manufacture of a vaccine for the prophylactics of infection with HIV in humans.

60. Use of a nucleotide sequence according to any of claims 14-55 for the manufacture of a composition for the treatment of an HIV infection in a human within 24 weeks of primary infection.

61. Use of the nucleotide sequence according to any of claims 14-55 for the production of a recombinant protein.

Amino acid	One letter amino acid code	Three letter amino acid code	Codon
Alanine	A	Ala	GCC
Arginine	R	Arg	CGC
Aspargine	N	Asn	AAC
Aspartic acid	D	Asp	GAC
Cysteine	C	Cys	TGC
Glutamine	Q	Gln	CAG
Glutamic acid	E	Glu	GAG
Glycine	G	Gly	GGC
Histidine	H	His	CAC
Isoleucine	I	Ile	ATC
Leucine	L	Leu	CTG
Lysine	K	Lys	AAG
Proline	P	Pro	CCC
Phenylalanine	F	Phe	TTC
Serine	S	Ser	AGC
Threonine	T	Thr	ACC
Tyrosine	Y	Tyr	TAC
Valine	V	Val	GTG

Fig. 1

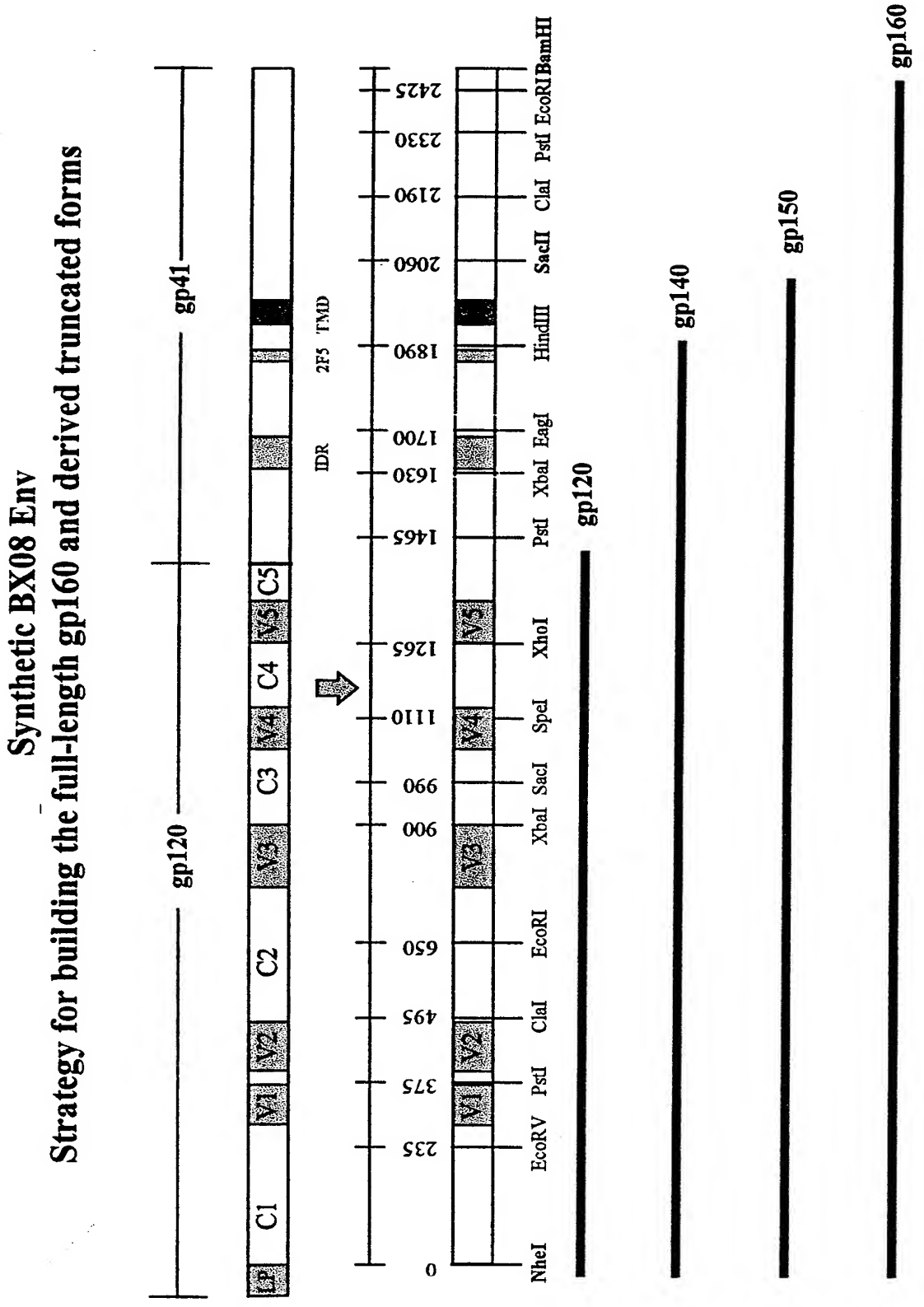
Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms
gp120
gp41
LP
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
IDR
2F5
TMD
NheI
EcoRV
PstI
ClaI
EcoRI
XbaI
SacI
SpeI
XhoI
PstI
XbaI
EagI
HindIII
SacII
ClaI
PstI
EcoRI
BamHI
0
235
375
495
650
900
990
1110
1265
1465
1630
1700
1890
2060
2190
2330
2425
gp120
gp140
gp150
gp160

Fig. 2

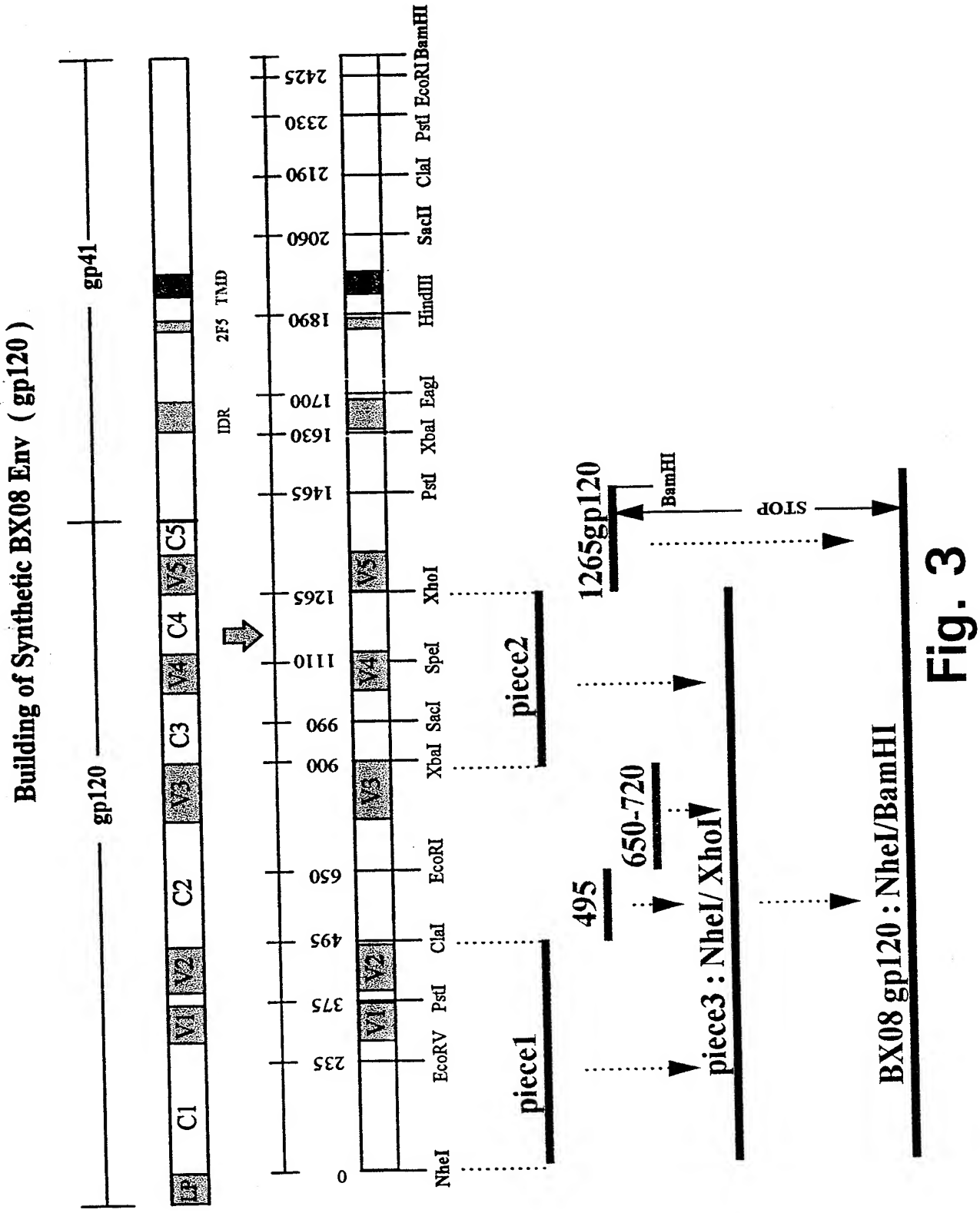
Building of Synthetic BX08 Env (gp120)
gp41
gp120
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
IDR
2F5
TMD
0
235
375
495
650
900
990
1110
1265
1465
1630
1700
1890
2060
2190
2330
2425
NheI
EcoRV
PstI
ClaI
EcoRI
XbaI
SacI
SpeI
XhoI
PstI
XbaI
EagI
HindIII
SacII
ClaI
PstI
EcoRI
BamHI
piece1
piece2
1265gp120
BamHI
STOP
495
650-720
piece3 : NheI/ XhoI
BX08 gp120 : NheI/BamHI

Fig. 3

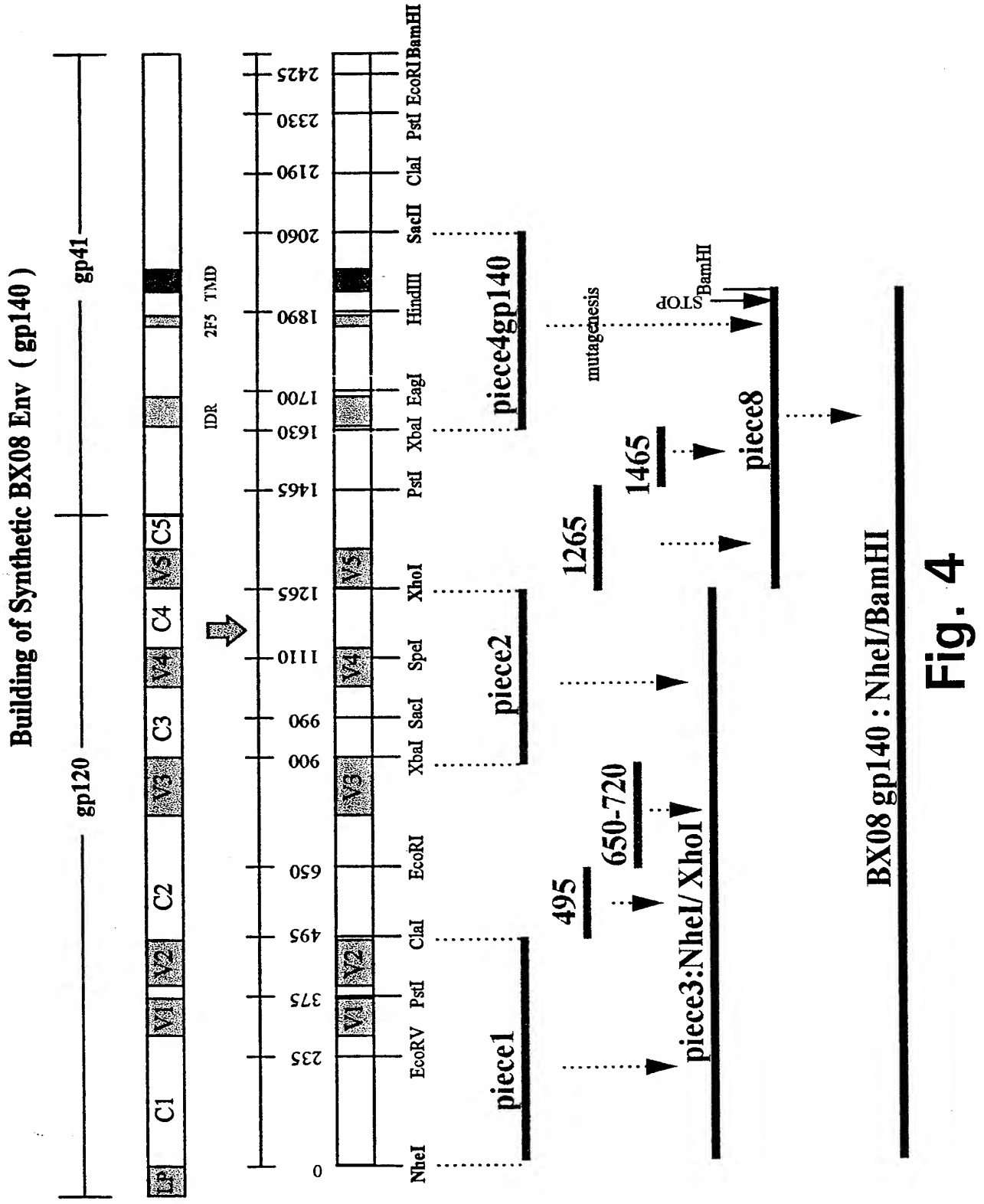
Building of Synthetic BX08 Env (gp140)
gp120
gp41
LP
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
IDR
2F5
TMD
0
235
375
495
650
900
990
1110
1265
1465
1630
1700
1890
2060
2190
2330
2425
NheI
EcoRV
PstI
ClaI
EcoRI
XbaI
SacI
SpeI
XhoI
PstI
XbaI
EagI
HindIII
SacII
ClaI
PstI
EcoRI
BamHI
piece1
piece2
piece4gp140
mutagenesis
STOP
BamHI
piece8
1465
1265
650-720
495
piece3:NheI/XhoI
BX08 gp140 : NheI/BamHI

Fig. 4

Fig. 4

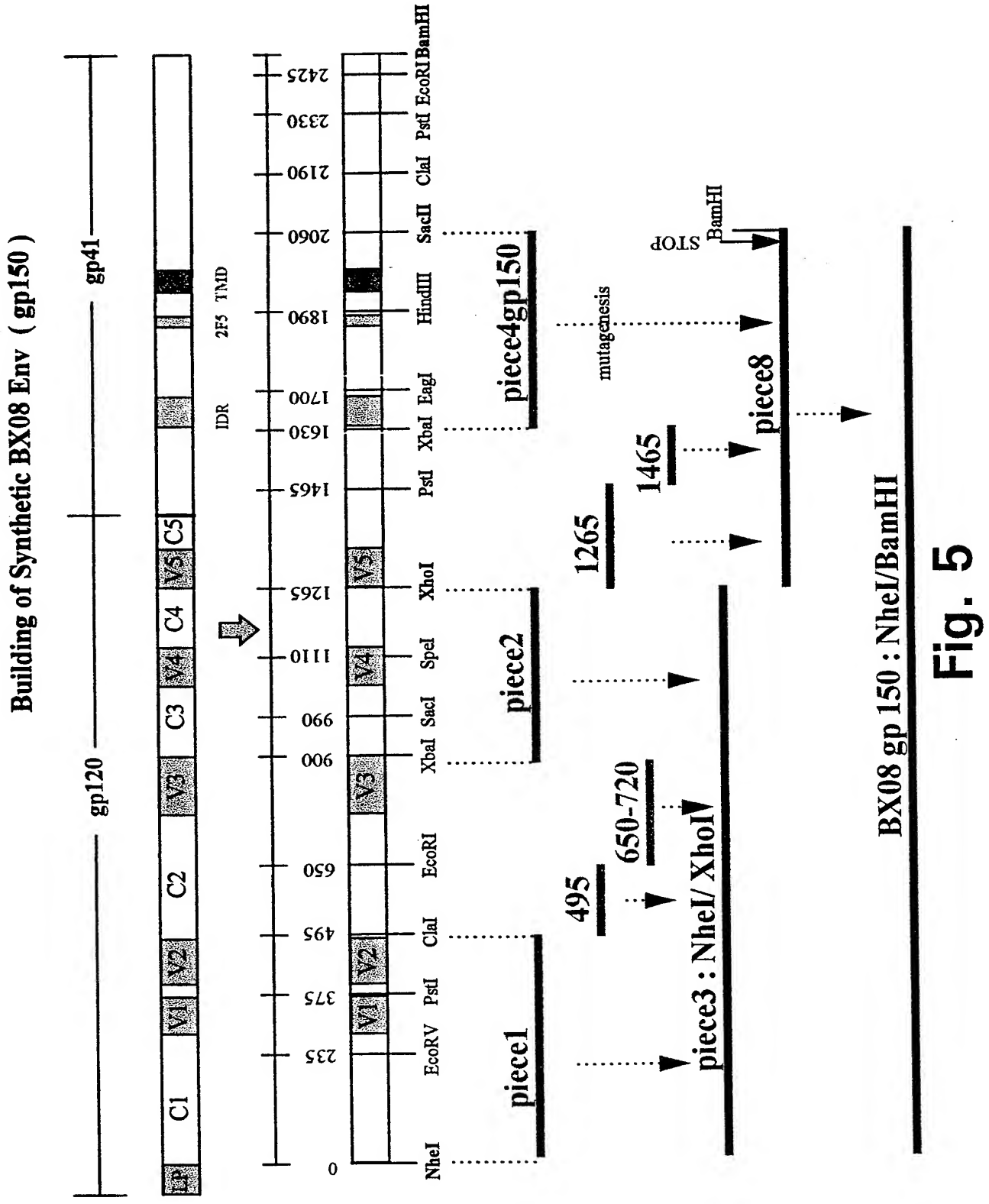
Building of Synthetic BX08 Env (gp150)
gp120
gp41
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
IDR
2F5
TMD
NheI
EcoRV
PstI
ClaI
EcoRI
XbaI
SacI
SpeI
XhoI
PstI
XbaI
EagI
HindIII
SacII
ClaI
PstI
EcoRI
BamHI
0
235
375
495
650
900
990
1110
1265
1630
1700
1890
2060
2190
2330
2425
piece1
piece2
piece4gp150
piece3 : NheI/ XhoI
495
650-720
1265
1465
mutagenesis
STOP
piece8
BX08 gp 150 : NheI/BamHI

Fig. 5

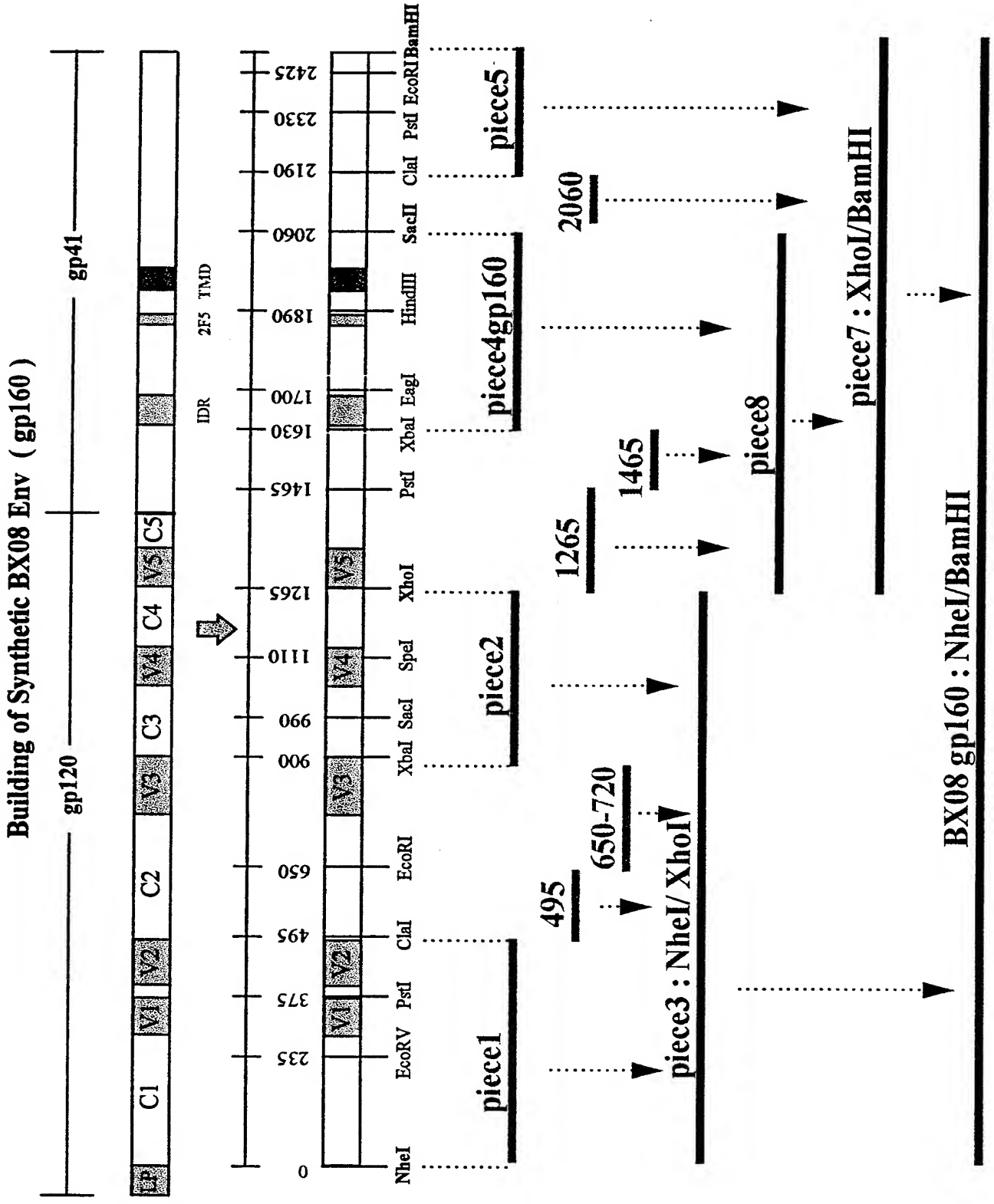
Building of Synthetic BX08 Env (gp160)
gp120
gp41
SP
C1
V1
V2
C2
V3
C3
V4
C4
V5
C5
IDR
2F5
TMD
0
235
375
495
650
900
990
1110
1265
1465
1630
1700
1890
2060
2190
2330
2425
NheI
EcoRV
PstI
ClaI
EcoRI
XbaI
SacI
SpeI
XhoI
PstI
XbaI
EagI
HindIII
SacII
ClaI
PstI
EcoRI
BamHI
piece1
piece2
piece4gp160
piece5
495
650-720
1265
1465
2060
piece3 : NheI/ XhoI
piece8
piece7 : XhoI/BamHI
BX08 gp160 : NheI/BamHI

Fig. 6

STARTS: ATG

aa		Σ	codons
A	Ala	GCX	GCT GCC GCG GCA
C	Cys	TGY	TGT TGC
D	Asp	GAY	GAT GAC
E	Glu	GAR	GAG GAA
F	Phe	TTY	TTT TTC
G	Gly	GGX	GGT GGC GGG GGA
H	His	CAY	CAT CAC
I	Ile	ATH	ATT ATC ATA
K	Lys	AAR	AAG AAA
L	Leu	YTX	TTG TTA CTT CTC CTG CTA
M	Met	ATG	ATG
N	Asn	AAY	AAT AAC
P	Pro	CCX	CCT CCC CCG CCA
Q	Gln	CAR	CAG CAA
R	Arg	MGX	CGT CGC CGG CGA AGG AGA
S	Ser	WSX	TCT TCC TCG TCA AGT AGC
T	Thr	ACX	ACT ACC ACG ACA
V	Val	GTX	GTT GTC GTG GTA
W	Trp	TGG	TGG
Y	Tyr	TAY	TAT TAC
.	•	TRR	TGA TAG TAA
X	?m		

5'	1st	2nd T	2nd C	2nd A	2nd G	3'	3rd
T	T	Phe	Ser	Tyr	Cys	T	T
		Phe	Ser	Tyr	Cys	C	C
		Leu	Ser	•	•	A	A
		Leu	Ser	•	Trp	G	G
C	T	Leu	Pro	His	Arg	T	T
		Leu	Pro	His	Arg	C	C
		Leu	Pro	Gln	Arg	A	A
		Leu	Pro	Gln	Arg	G	G
A	T	Ile	Thr	Asn	Ser	T	T
		Ile	Thr	Asn	Ser	C	C
		Ile	Thr	Lys	Arg	A	A
		› Met	Thr	Lys	Arg	G	G
G	T	Val	Ala	Asp	Gly	T	T
		Val	Ala	Asp	Gly	C	C
		Val	Ala	Glu	Gly	A	A
		Val	Ala	Glu	Gly	G	G

Fig. 7

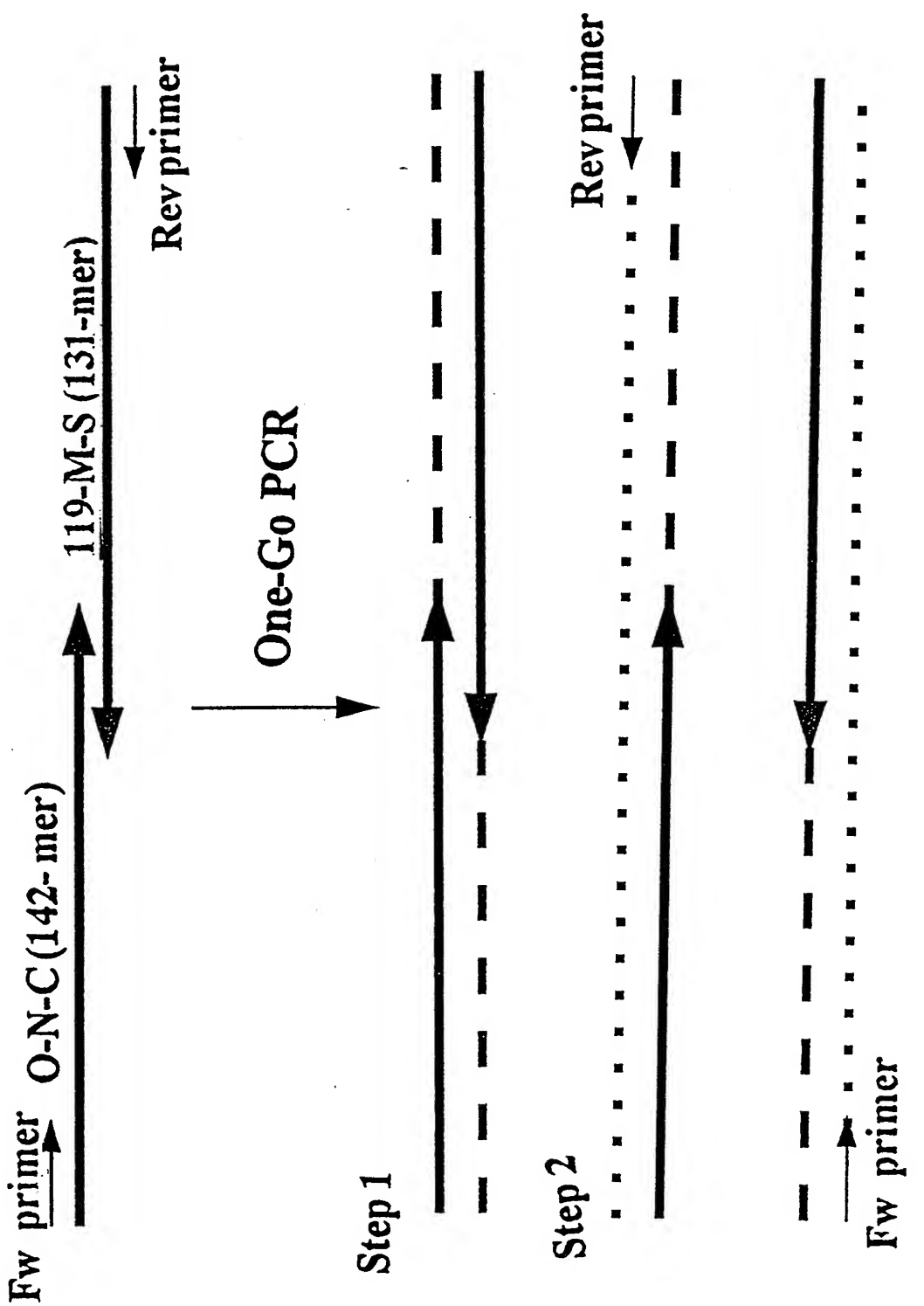
Overlapping PCR Strategy: Snut O-N-LANG (249 bases)
Fw primer
O-N-C (142- mer)
119-M-S (131-mer)
Rev primer
One-Go PCR
Step 1
Step 2
Rev primer
Fw primer

Fig. 8

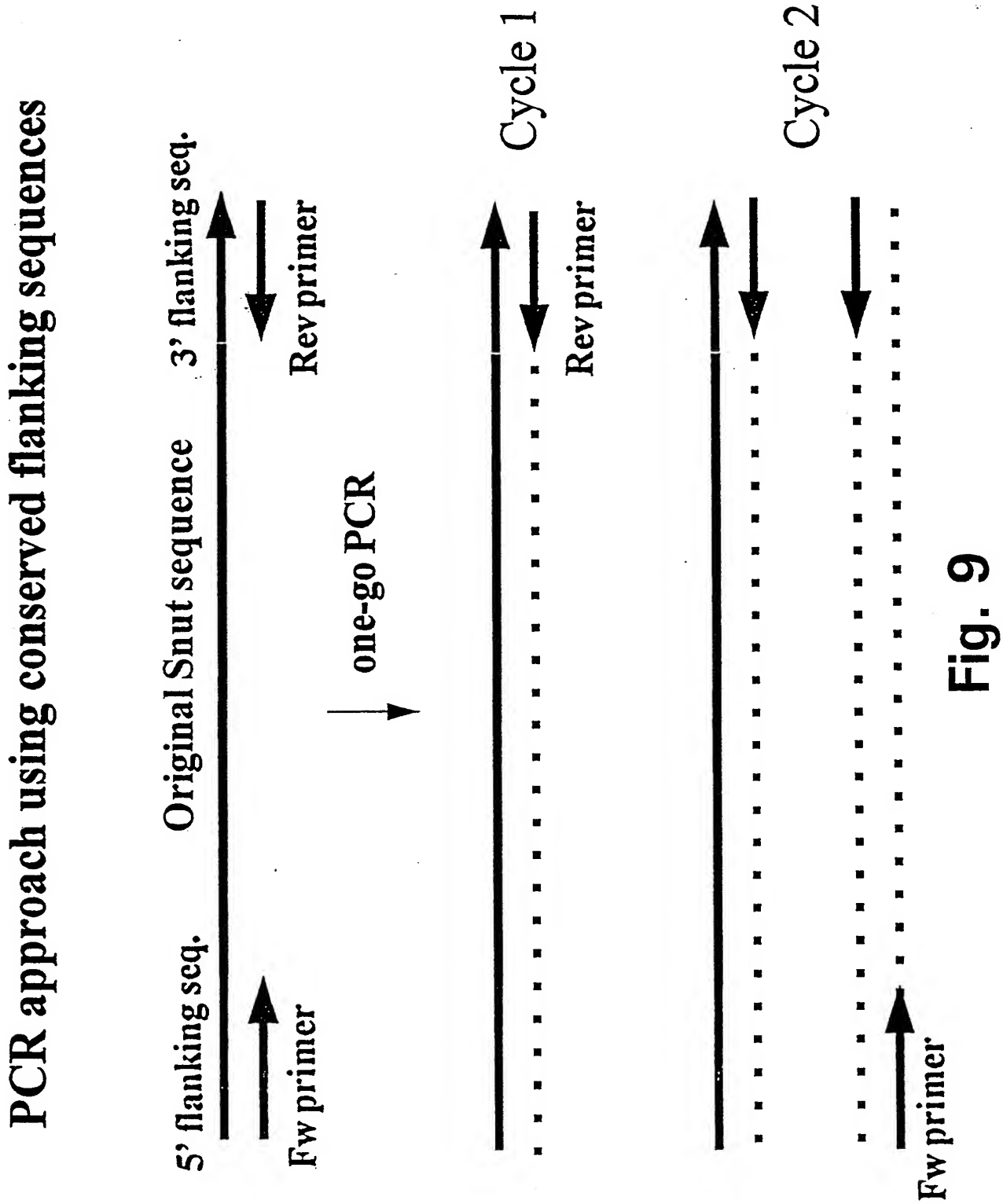
PCR approach using conserved flanking sequences
5' flanking seq.
Original Snut sequence
3' flanking seq.
Fw primer
Rev primer
one-go PCR
Cycle 1
Rev primer
Cycle 2
Fw primer

Fig. 9

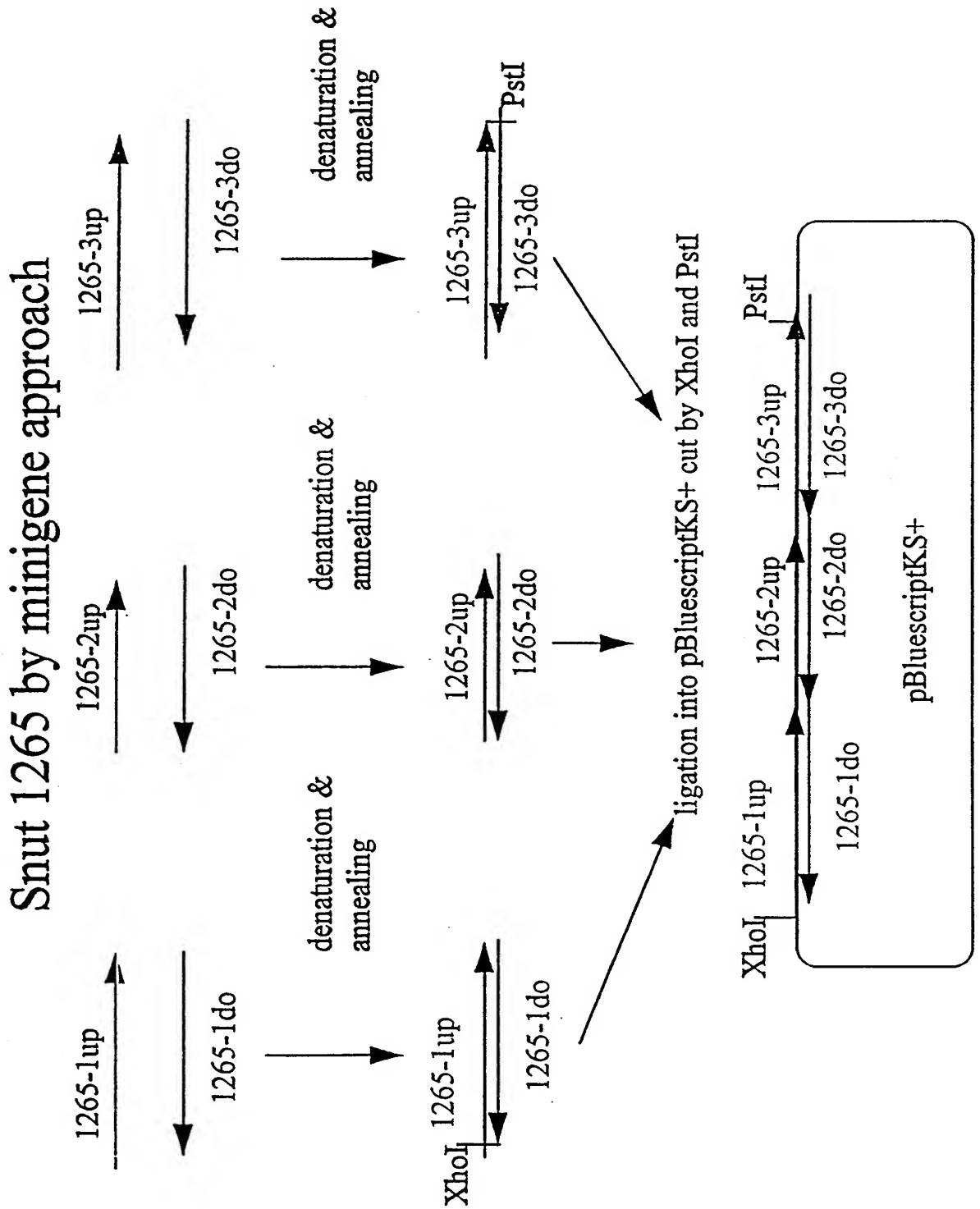
Snut 1265 by minigene approach
1265-1up
1265-1do
1265-2up
1265-2do
1265-3up
1265-3do
denaturation & annealing
denaturation & annealing
denaturation & annealing
XhoI
1265-1up
1265-1do
1265-2up
1265-2do
1265-3up
1265-3do
PstI
ligation into pBluescriptKS+ cut by XhoI and PstI
XhoI
1265-1up
1265-2up
1265-3up
PstI
1265-1do
1265-2do
1265-3do
pBluescriptKS+

Fig. 10

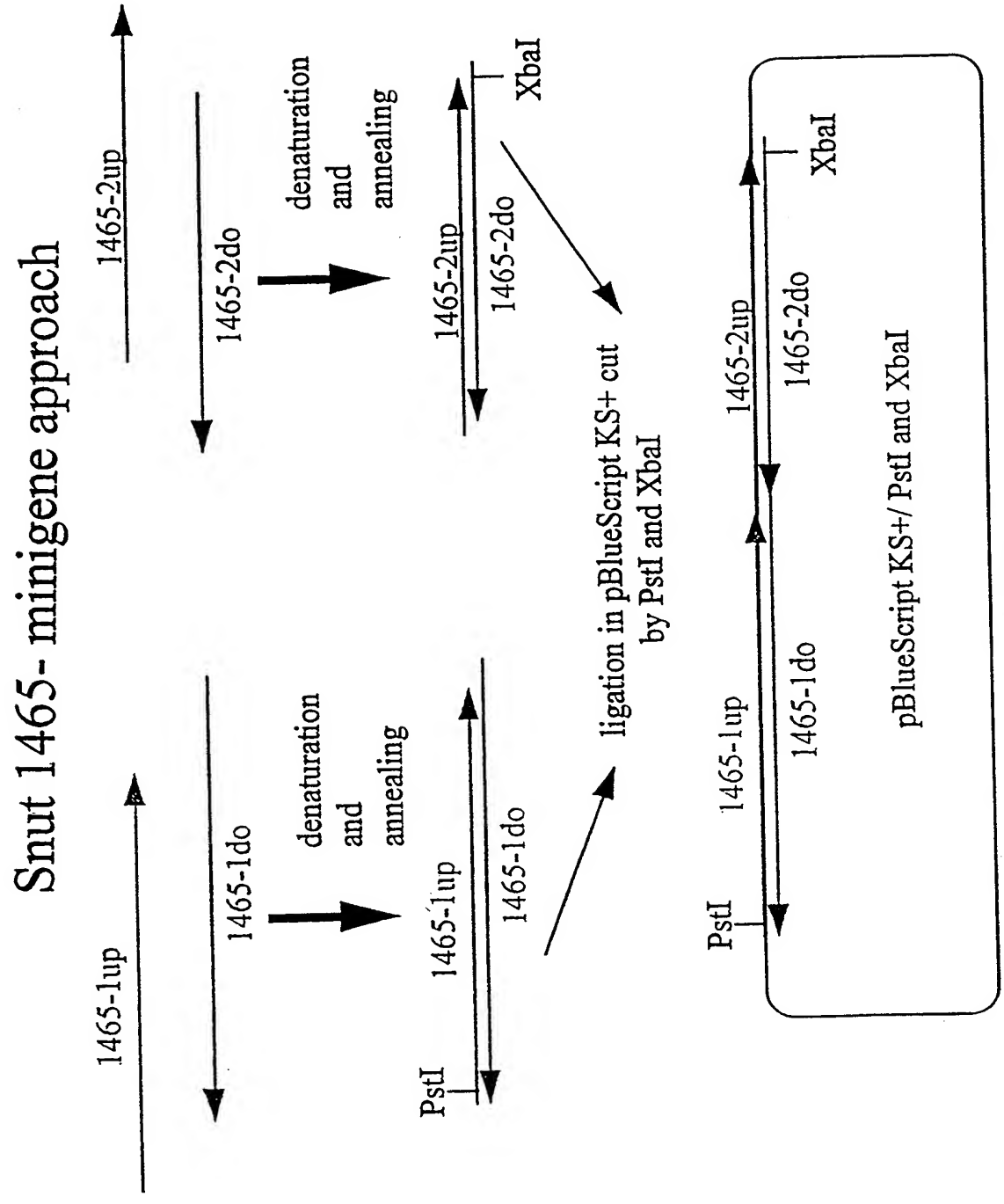
Snut 1465- minigene approach
1465-1up
1465-1do
denaturation and annealing
1465-1up
1465-1do
PstI
1465-2up
1465-2do
denaturation and annealing
1465-2up
1465-2do
XbaI
ligation in pBlueScript KS+ cut by PstI and XbaI
PstI
1465-1up
1465-1do
1465-2up
1465-2do
XbaI
pBlueScript KS+/ PstI and XbaI

Fig. 11

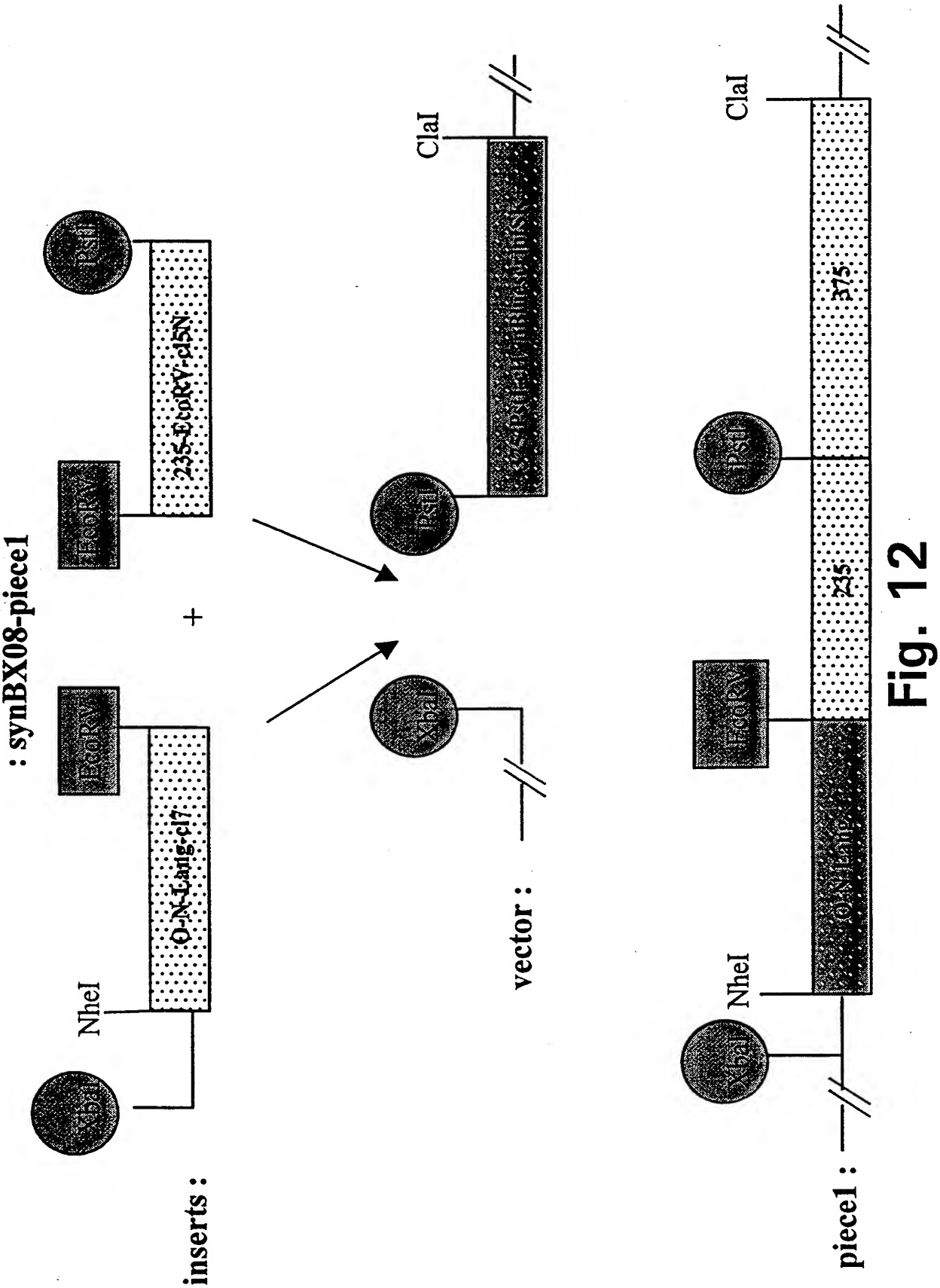
: synBX08-piece1
inserts :
XbaI
NheI
O-N-Lang-cl7
EcoRV
PstI
235-EcoRV-cl5N
+
vector :
XbaI
PstI
ClaI
piece1 :
XbaI
NheI
O-N-Lang-cl7
EcoRV
PstI
235
375
ClaI

Fig. 12

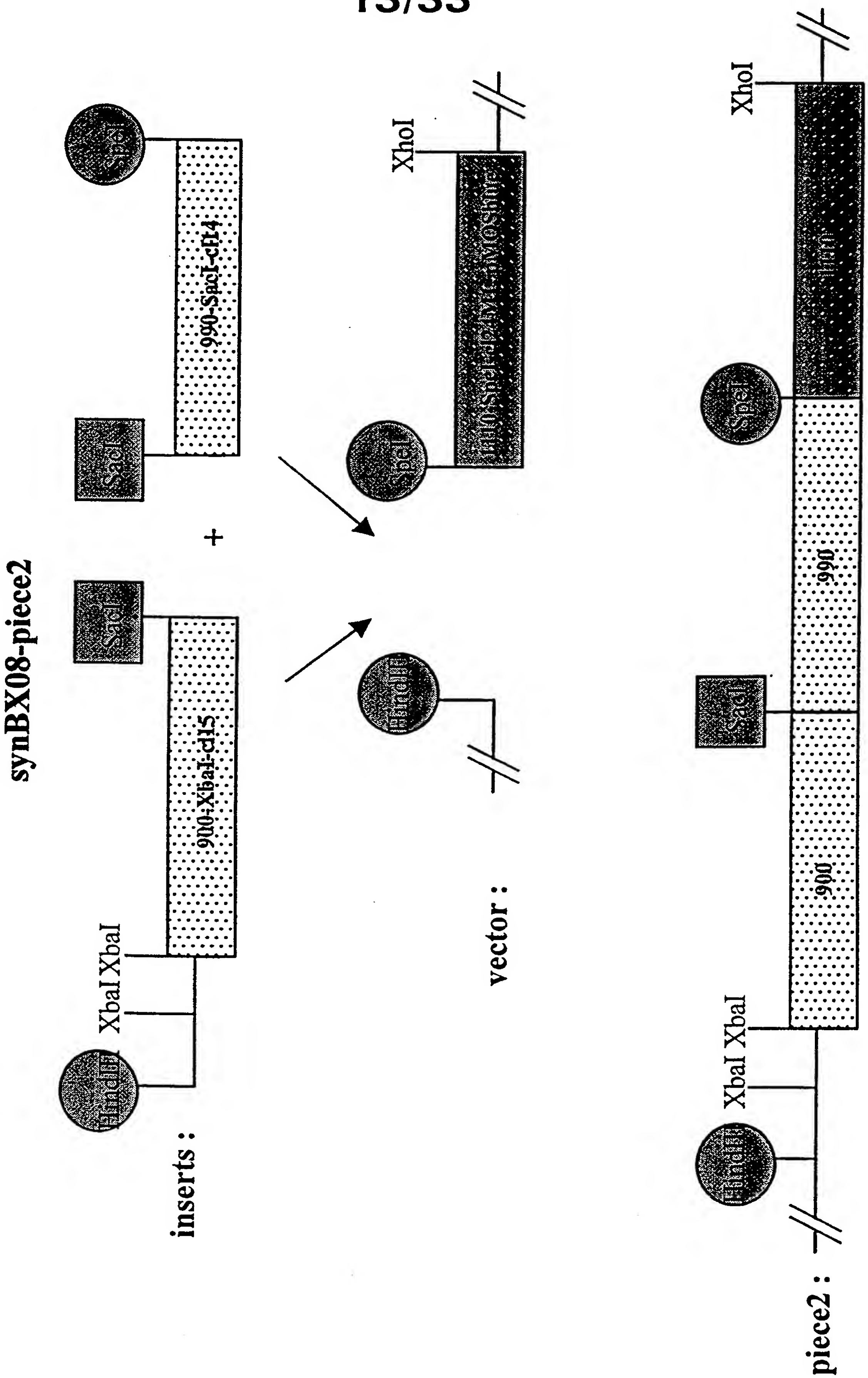
synBX08-piece2
inserts :
HindIII
XbaI XbaI
900-XbaI-cl15
SacI
+
SacI
990-SacI-cl14
SpeI
vector :
HindIII
SpeI
XhoI
piece2 :
HindIII
XbaI XbaI
900
SacI
990
SpeI
XhoI

Fig. 13

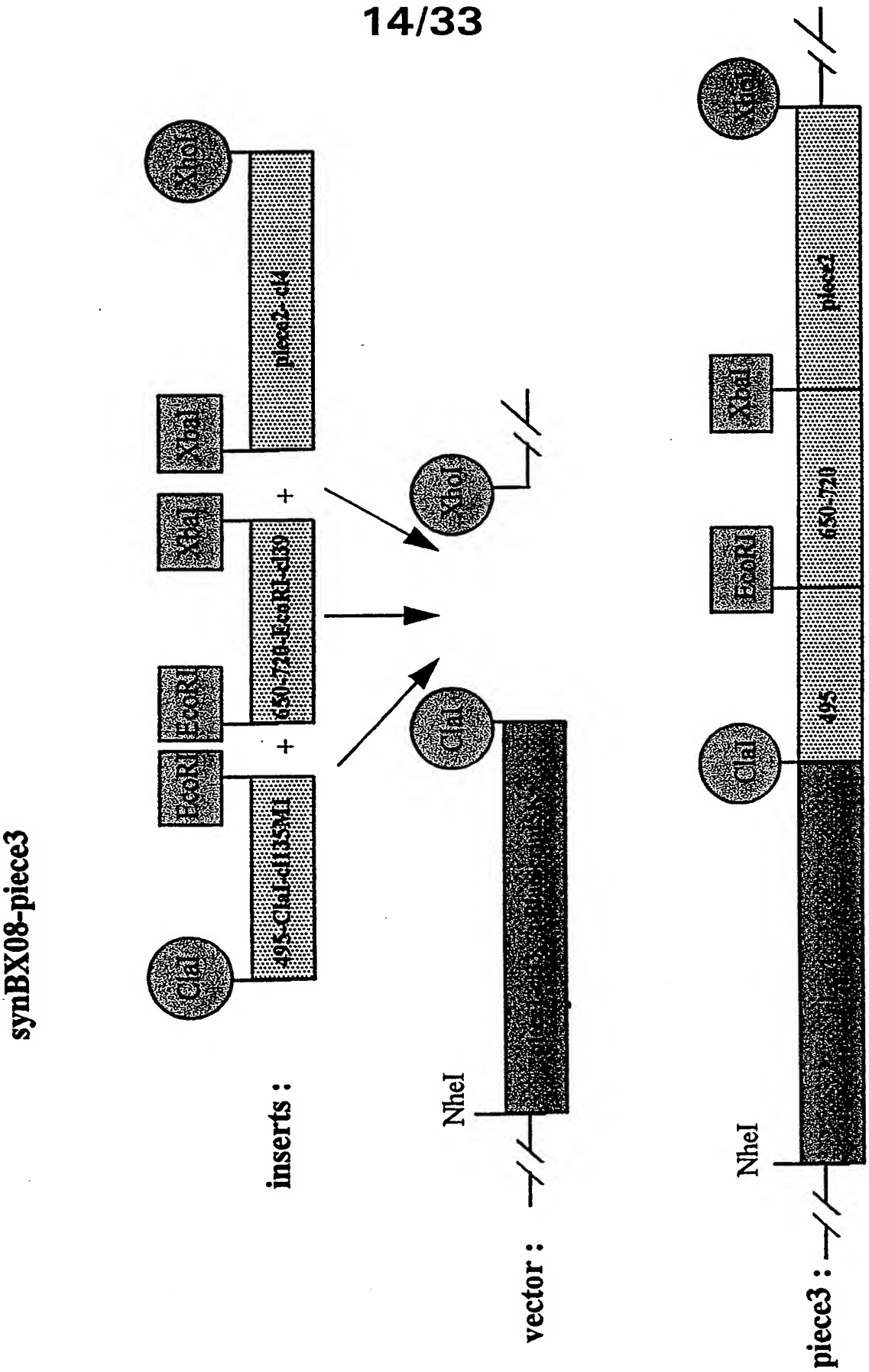
synBX08-piece3
inserts :
ClaI
495-ClaI-clBSMI
EcoRI
EcoRI
650-728-EcoRI-3BS
XbaI
XbaI
piece2- c4
XbaI
vector :
ClaI
NheI
piece3 :
ClaI
495
EcoRI
650-720
XbaI
piece3
XbaI
NheI

Fig. 14

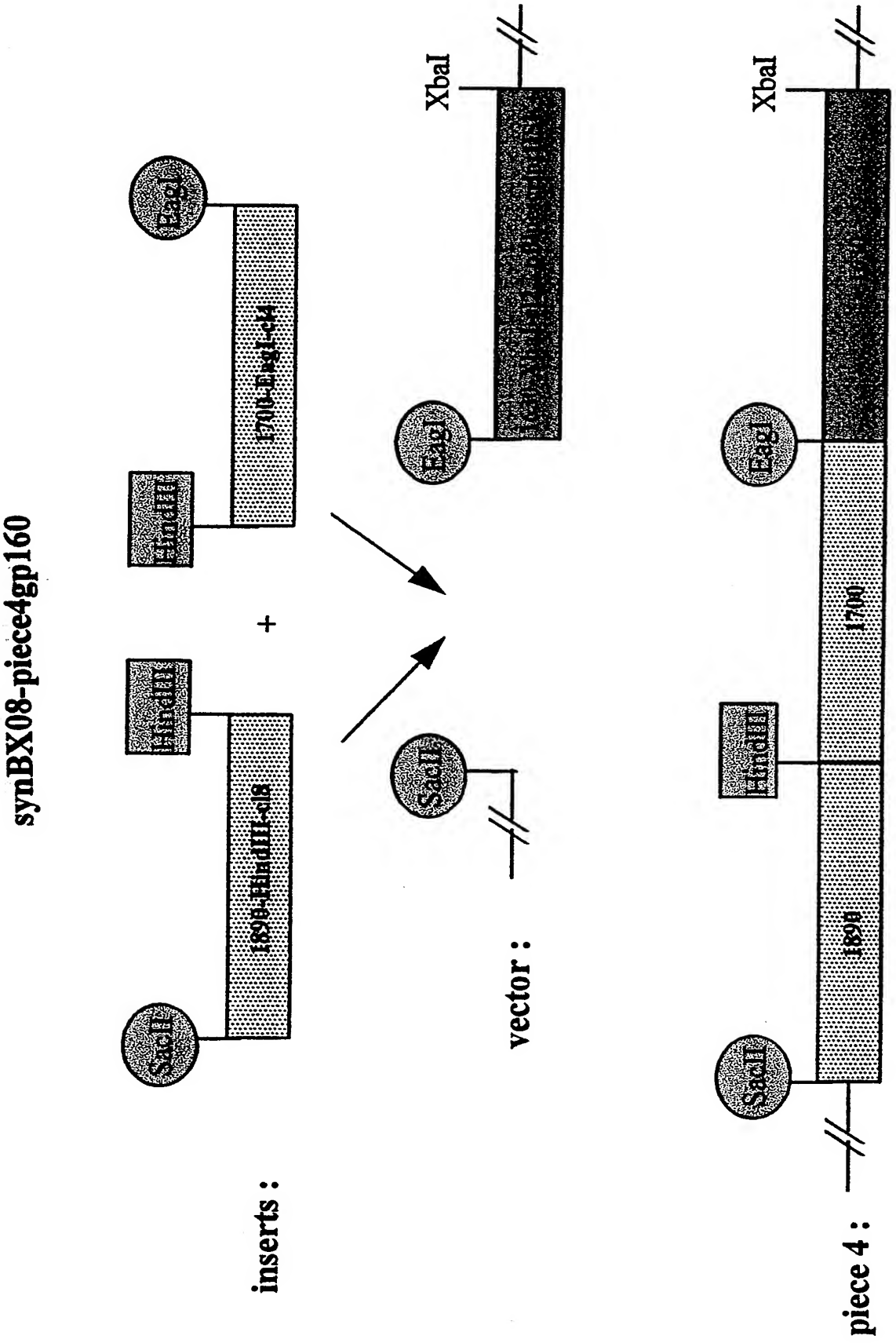
synBX08-piece4gp160
inserts :
SacII
1898-HindIII-c18
HindIII
+
EagI
HindIII
1700-EagI-c4
vector :
SacII
EagI
XbaI
piece 4 :
SacII
1898
HindIII
1700
EagI
XbaI

Fig. 15

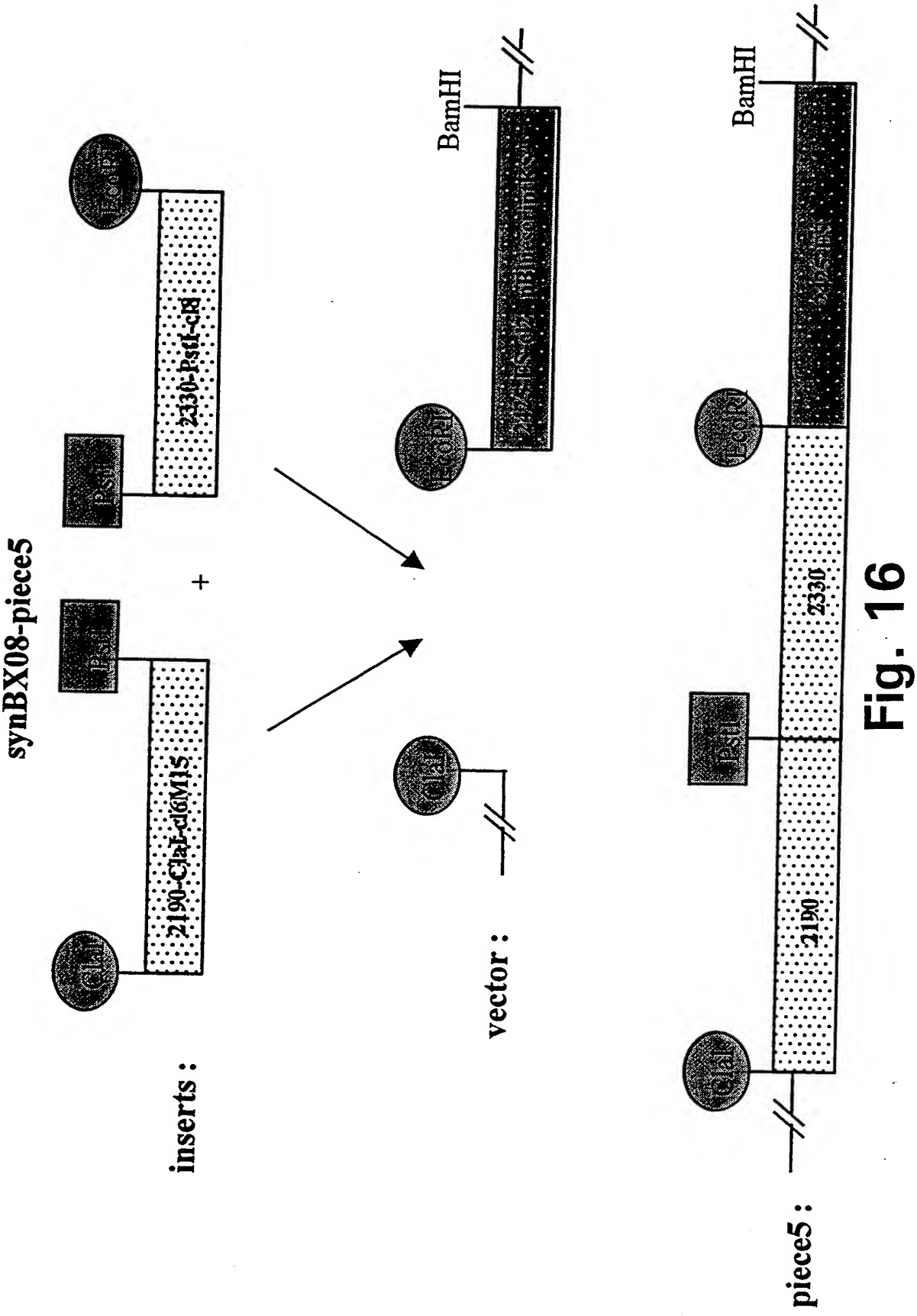
synBX08-piece5
inserts :
ClaI
2190-ClaI-cl6M15
PstI
+
PstI
2330-PstI-cl8
EcoRI
vector :
ClaI
EcoRI
BamHI
piece5 :
ClaI
2190
PstI
2330
EcoRI
BamHI

Fig. 16

Figure 17 : synBX08-piece8gp160

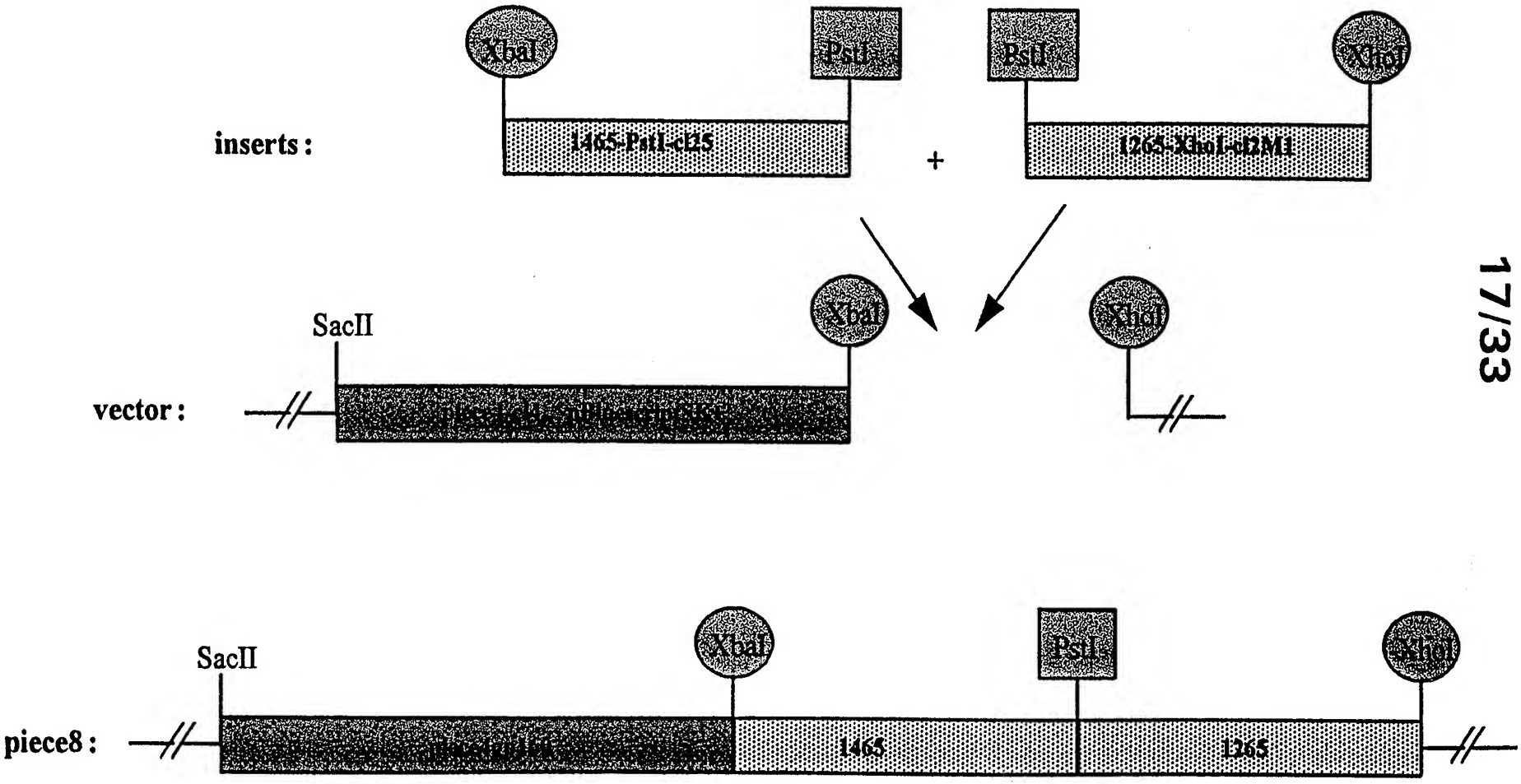

Fig. 17

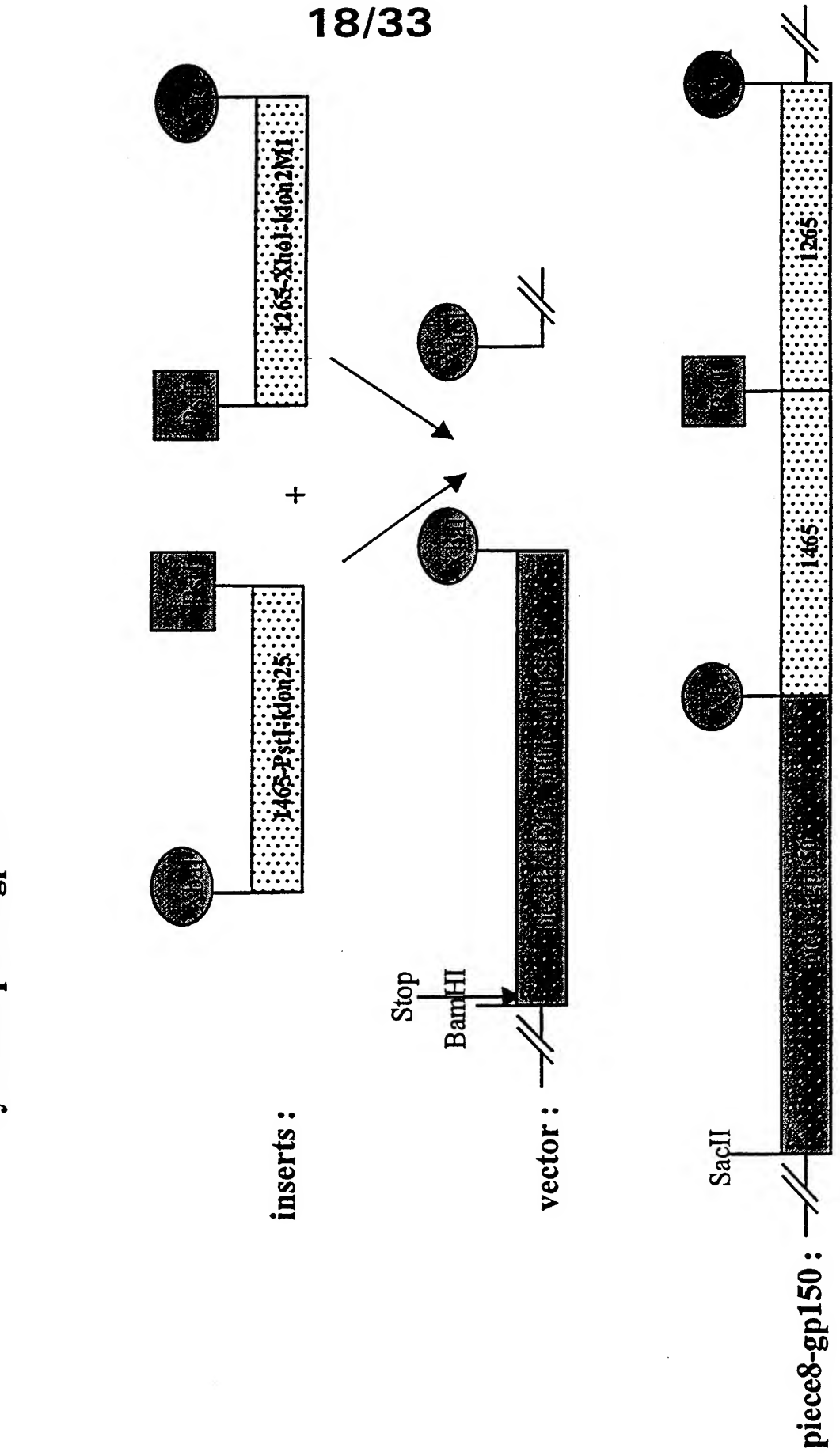
synBX08-piece8-gp150
inserts :
1465-PstI-Kon25
1265-XhoI-Kon2M1
+
vector :
Stop
BamHI
piece8-gp150 :
SacII
1465
1265

Fig. 18

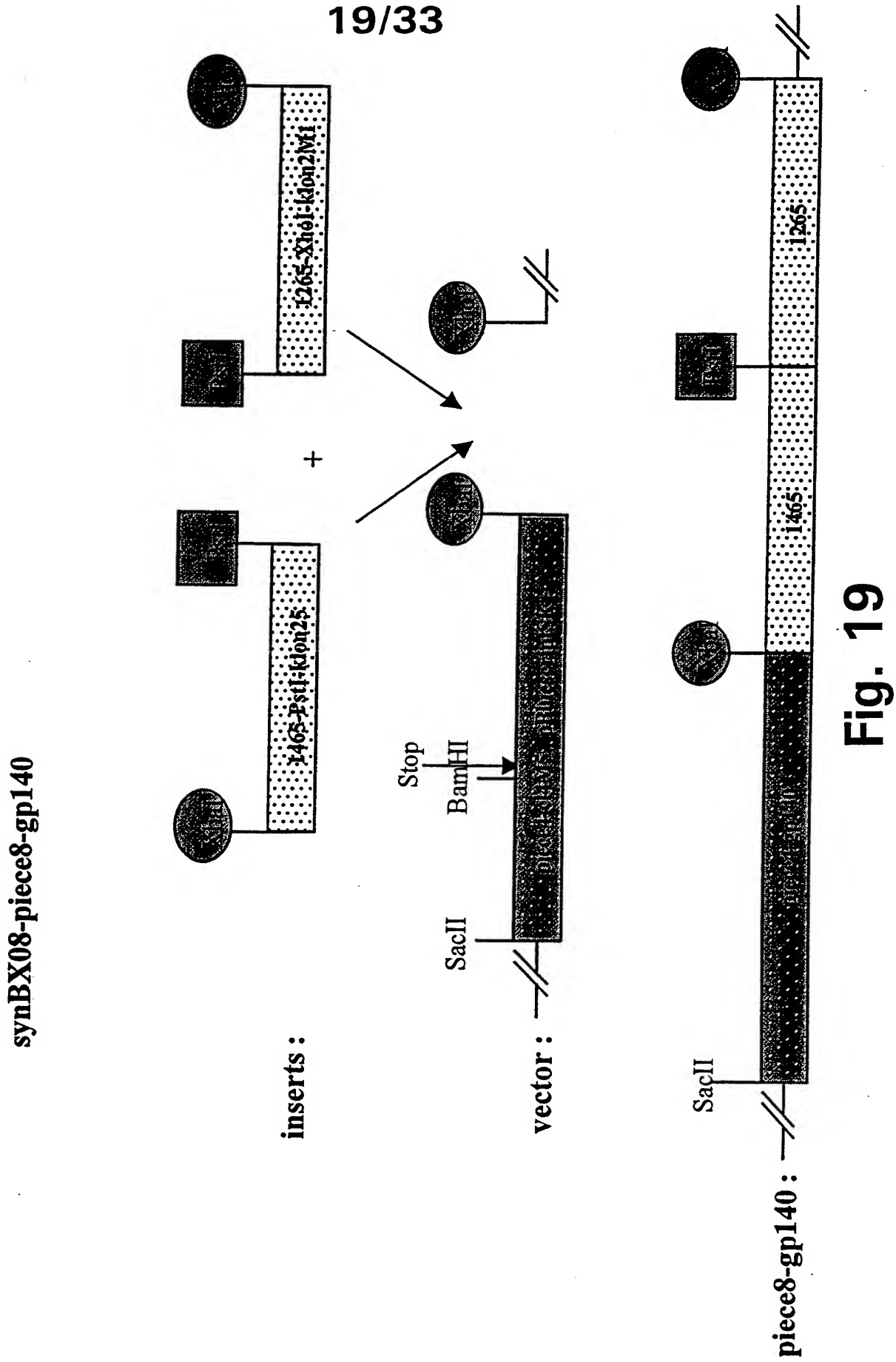
synBX08-piece8-gp140
inserts :
1465-PstI-kon25
1265-XhoI-kon2Mt1
+
vector :
Stop
BamHI
SacII
piece8-gp140 :
SacII
1465
1265

Fig. 19

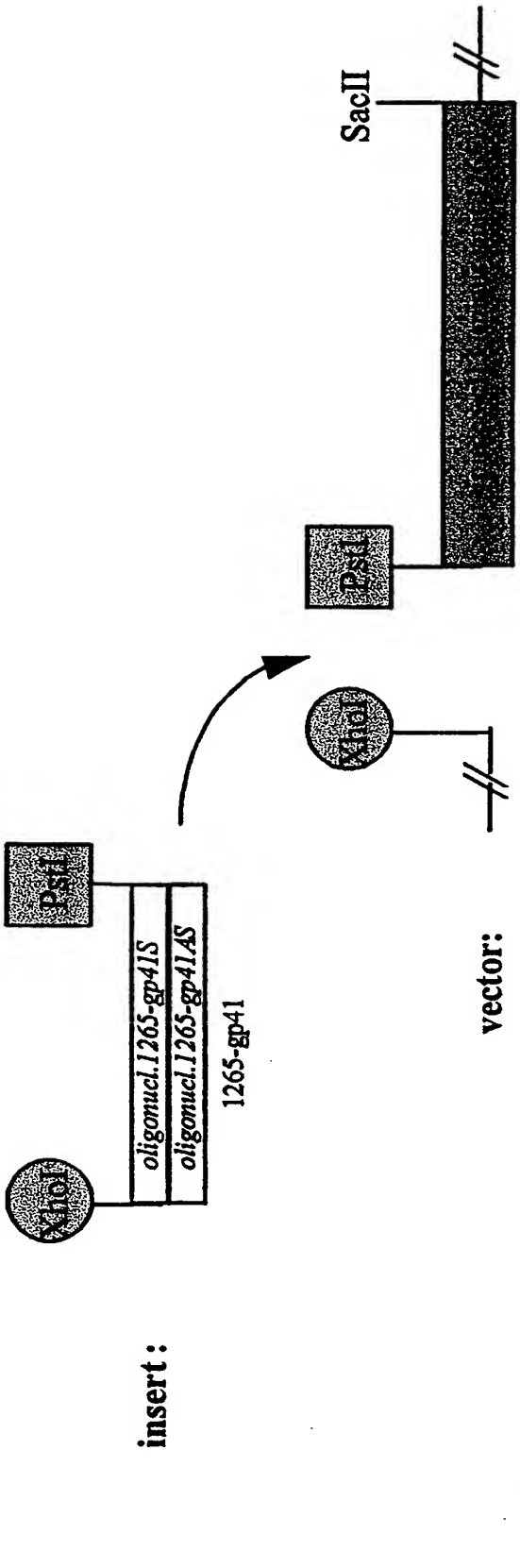

Figure 20: synBX08-piece8-gp41

Fig. 20

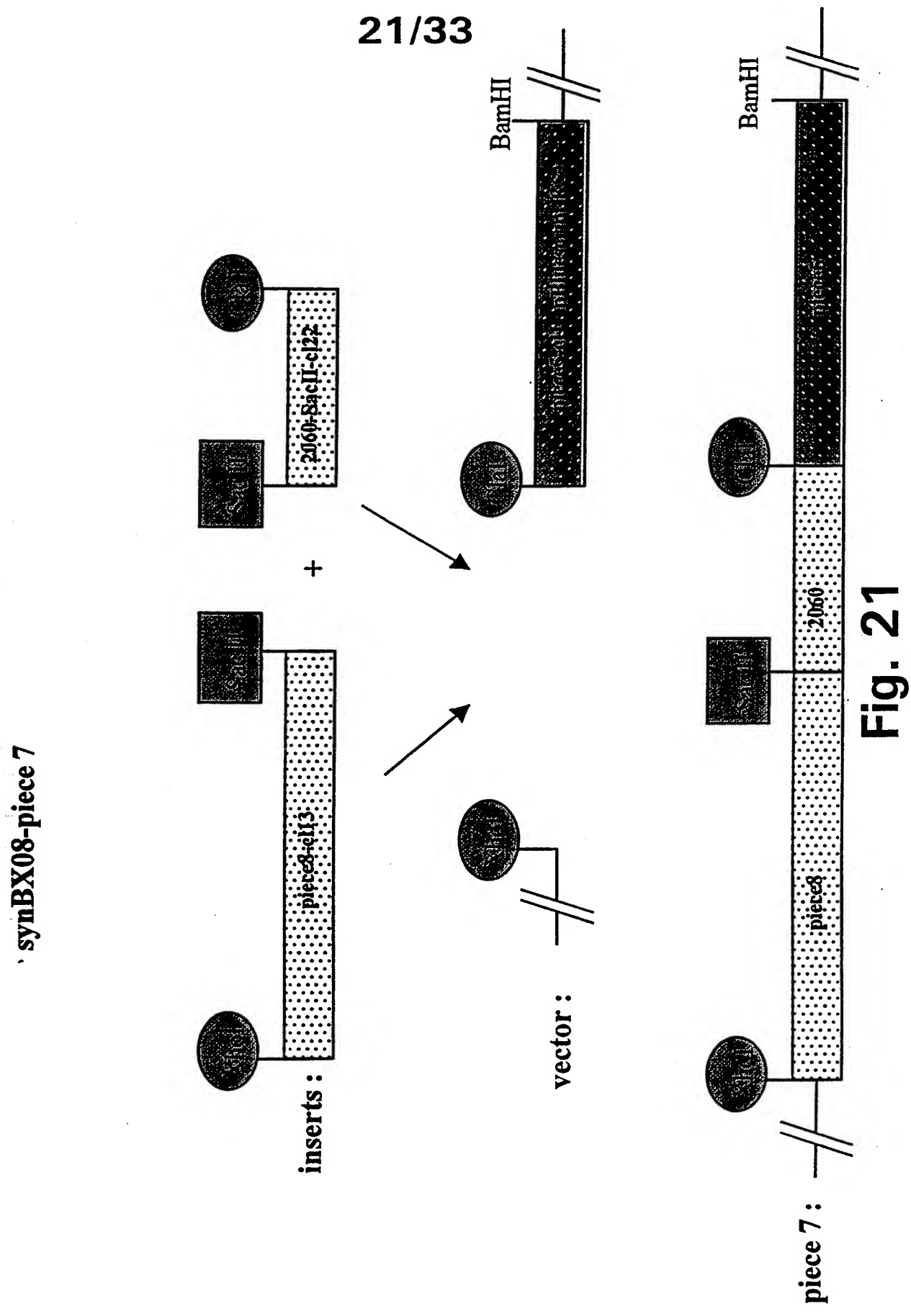
synBX08-piece 7
inserts :
piece8-cl13
2060-SacII-cl22
+
vector :
BamHI
piece 7 :
2060
piece8
BamHI

Fig. 21

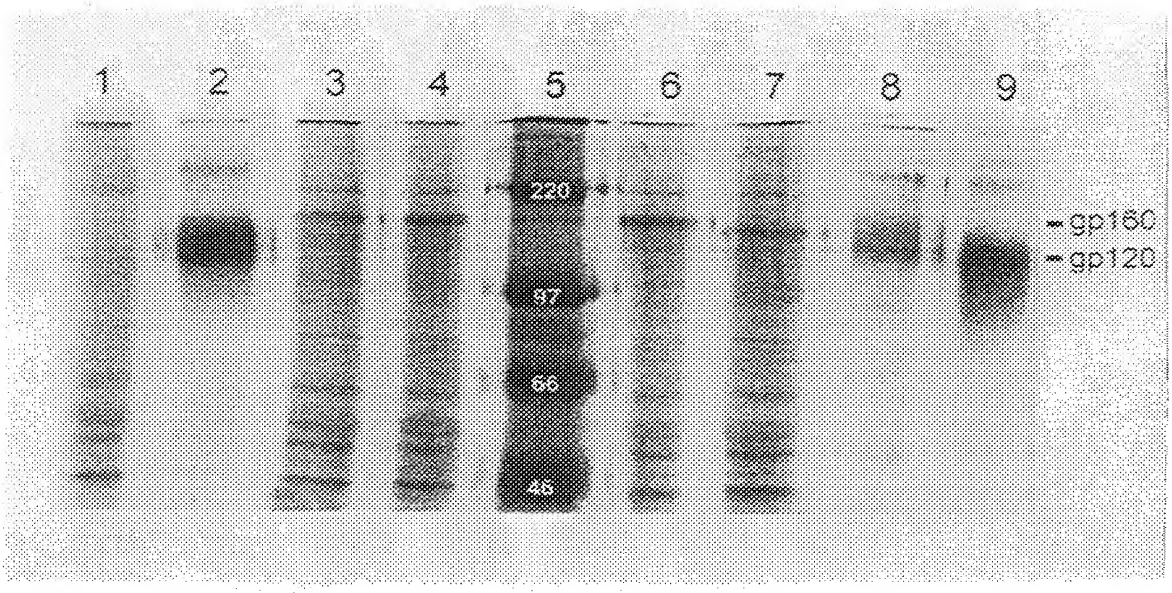

Fig. 22 A

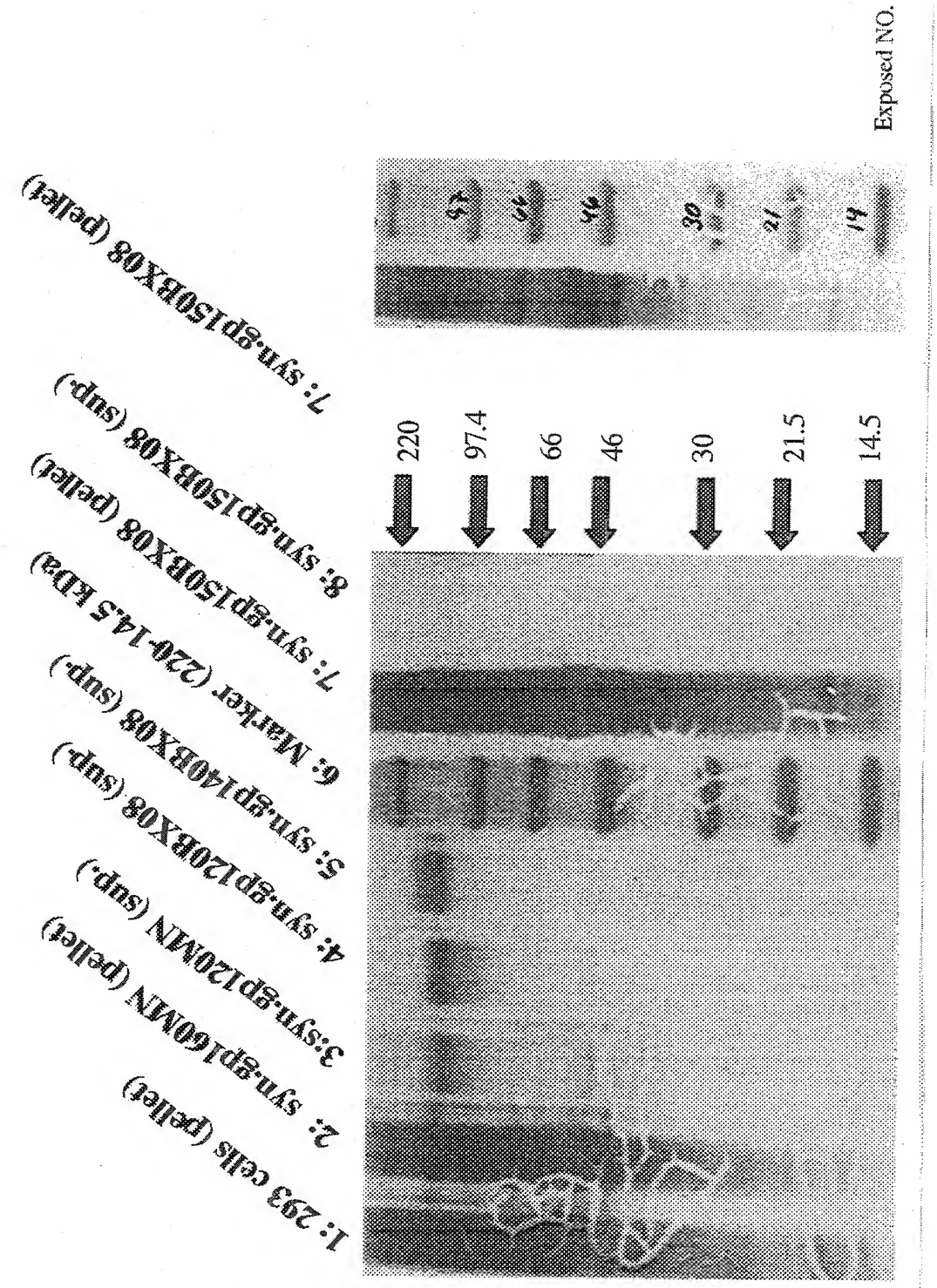
1: 293 cells (pelled)
2: syn.gp160MN (pelled)
3:syn.gp120MN (sup.)
4: syn.gp120BX08 (sup.)
5: syn.gp140BX08 (sup.)
6: Marker (220-14.5 kDa)
7: syn.gp150BX08 (pelled)
8: syn.gp150BX08 (sup.)
7: syn.gp150BX08 (pelled)
220
97.4
66
46
30
21.5
14.5
Exposed NO.

Fig. 22 B

Panel A

Fig. 22 C

Panel B

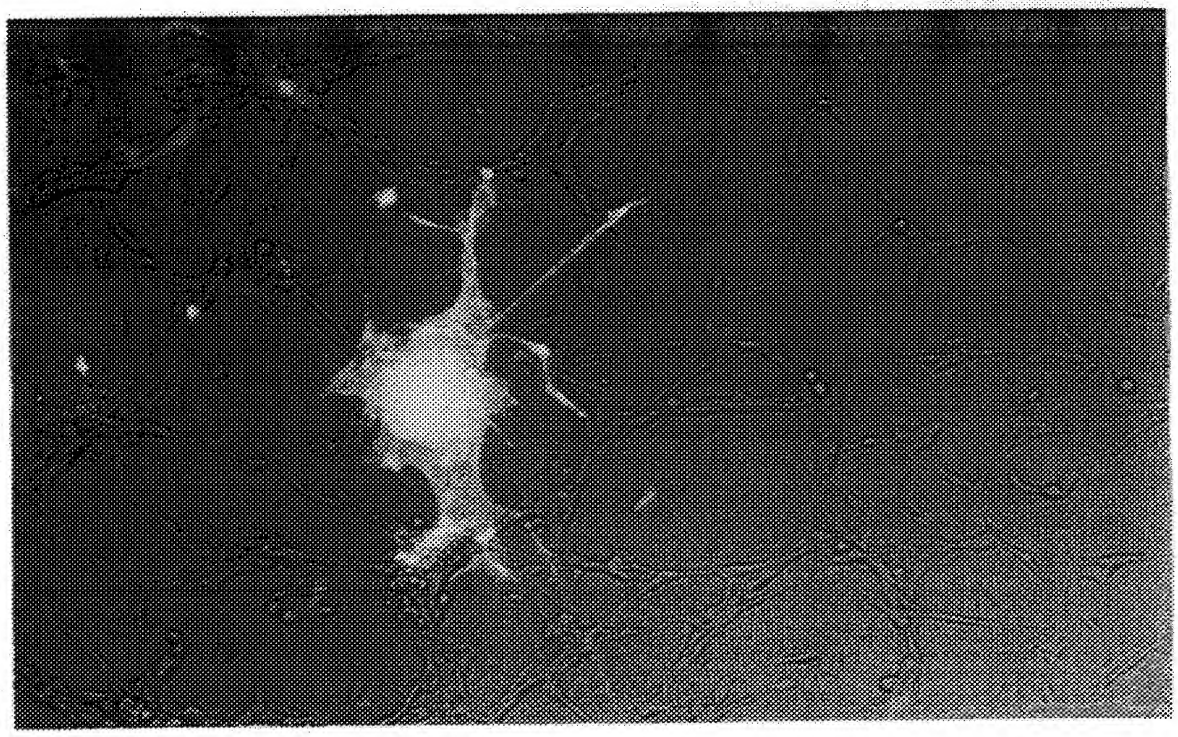

Fig. 22 C

Panel C

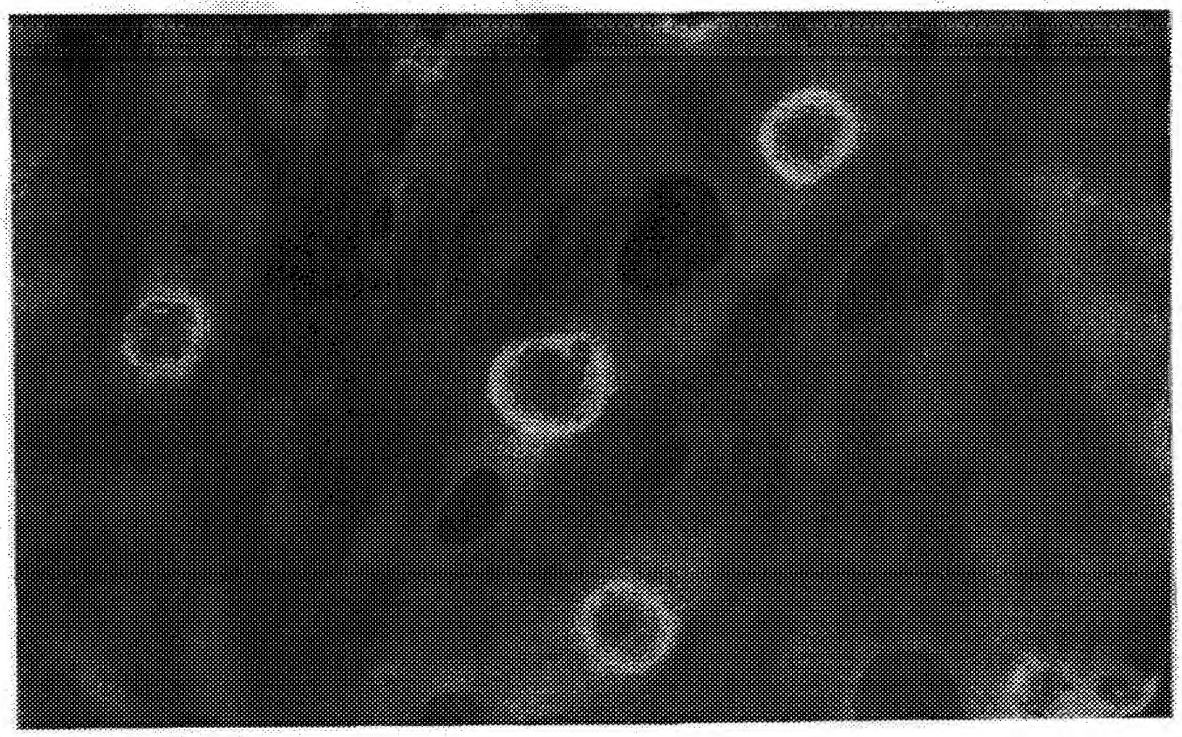

Fig. 22 C

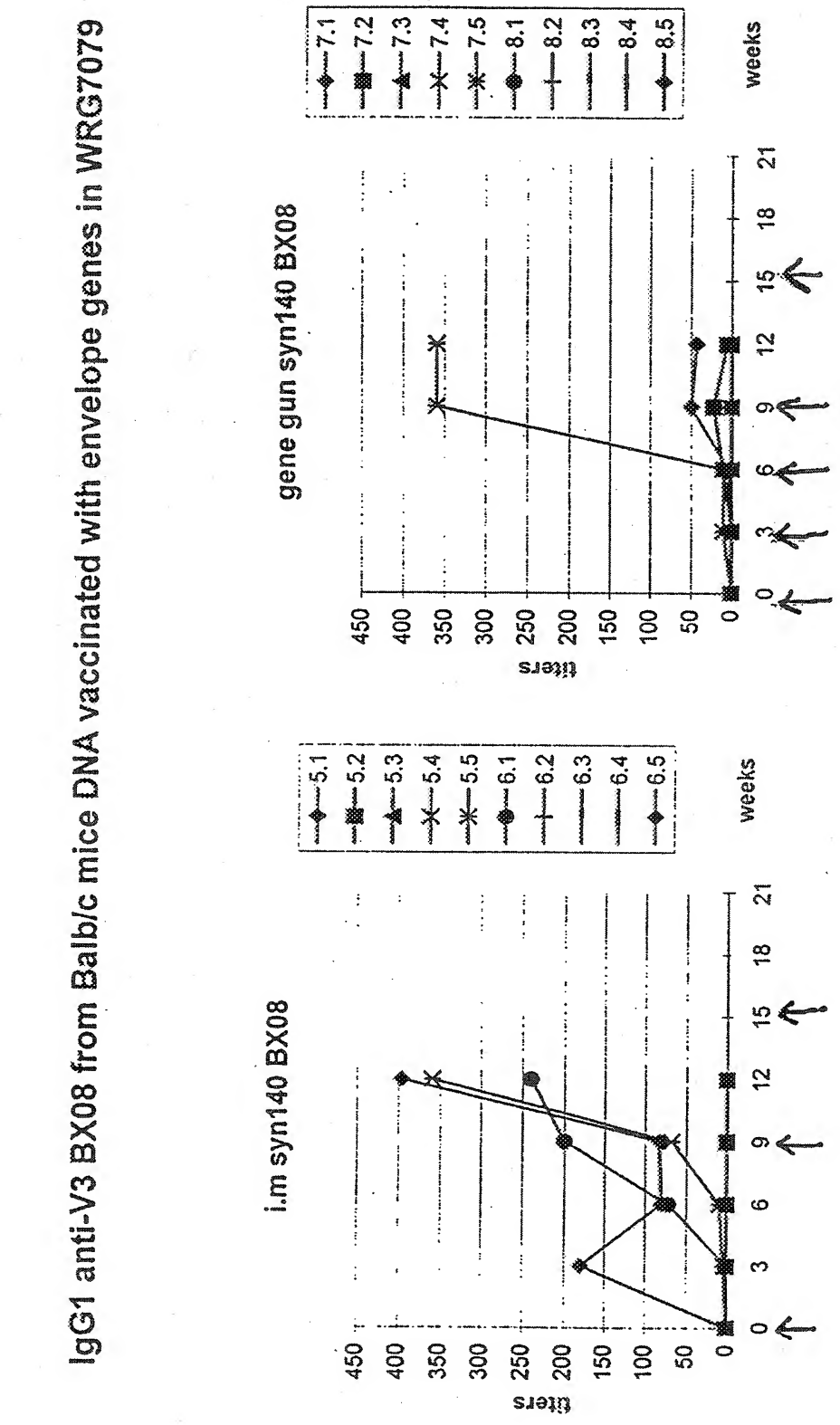
IgG1 anti-V3 BX08 from Balb/c mice DNA vaccinated with envelope genes in WRG7079
i.m syn140 BX08
gene gun syn140 BX08
titers
weeks
0
3
6
9
12
15
18
21
50
100
150
200
250
300
350
400
450
5.1
5.2
5.3
5.4
5.5
6.1
6.2
6.3
6.4
6.5
7.1
7.2
7.3
7.4
7.5
8.1
8.2
8.3
8.4
8.5

Fig. 23

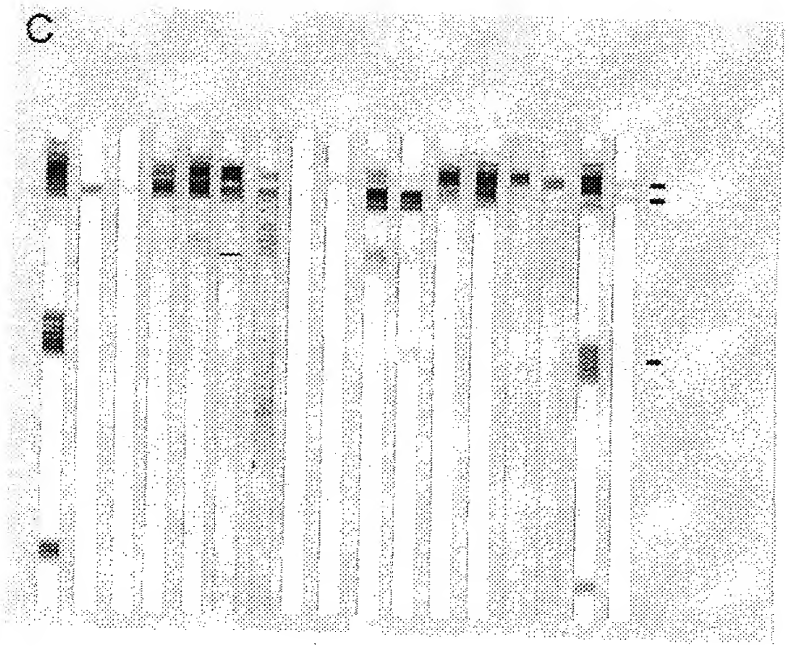

Fig. 24

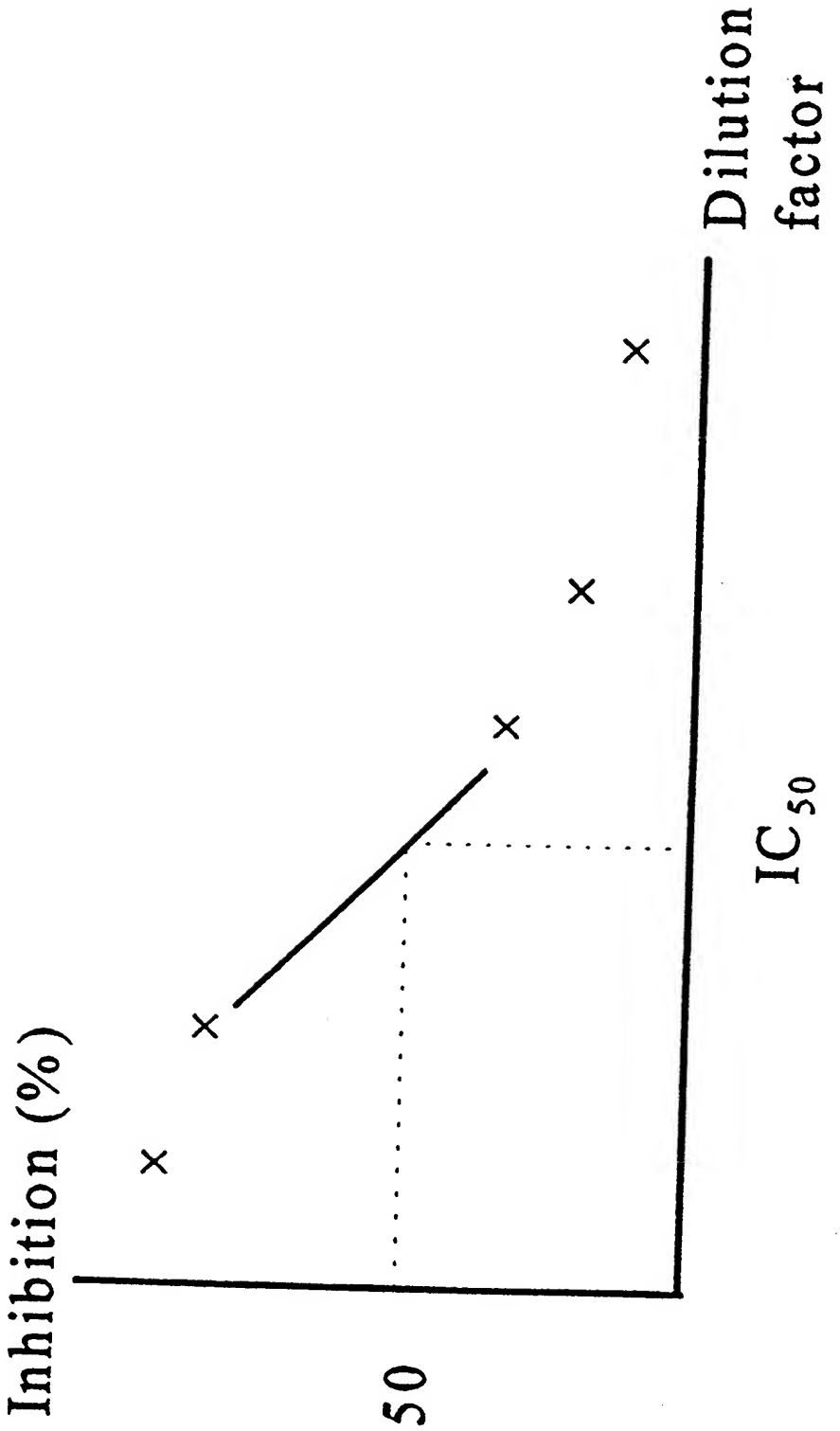

Fig. 25

Fig. 26 A

CTL activity induced by different synthetic DNA vaccines

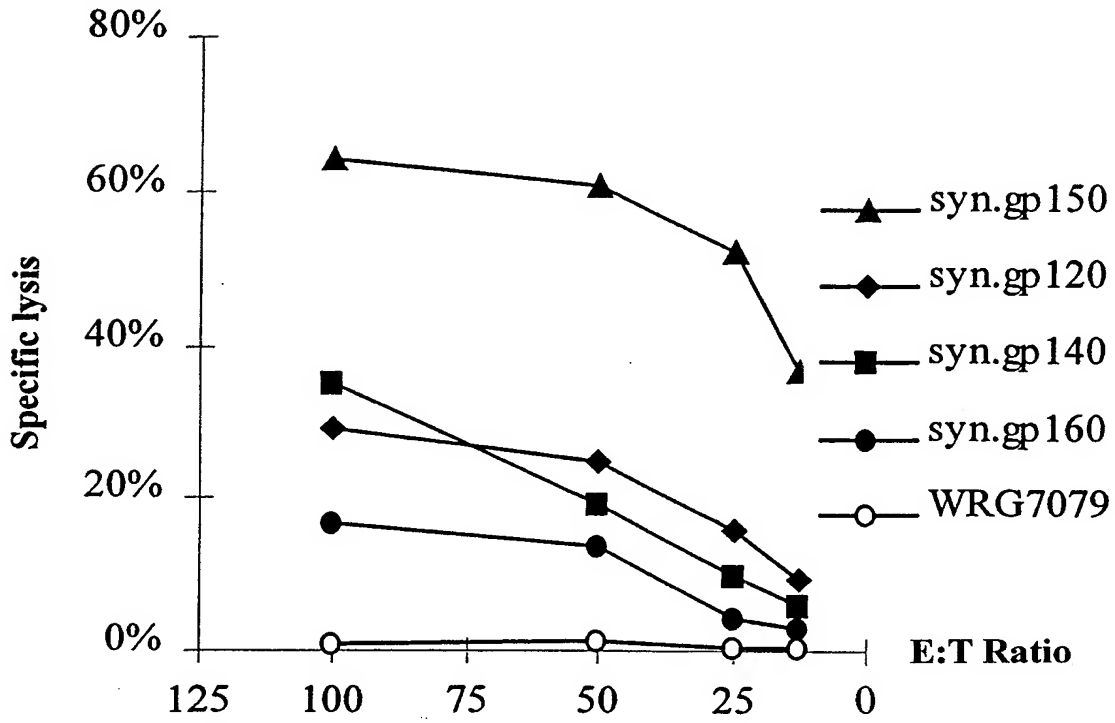

Fig. 26 B

CTL activity induced by different DNA delivery methods.

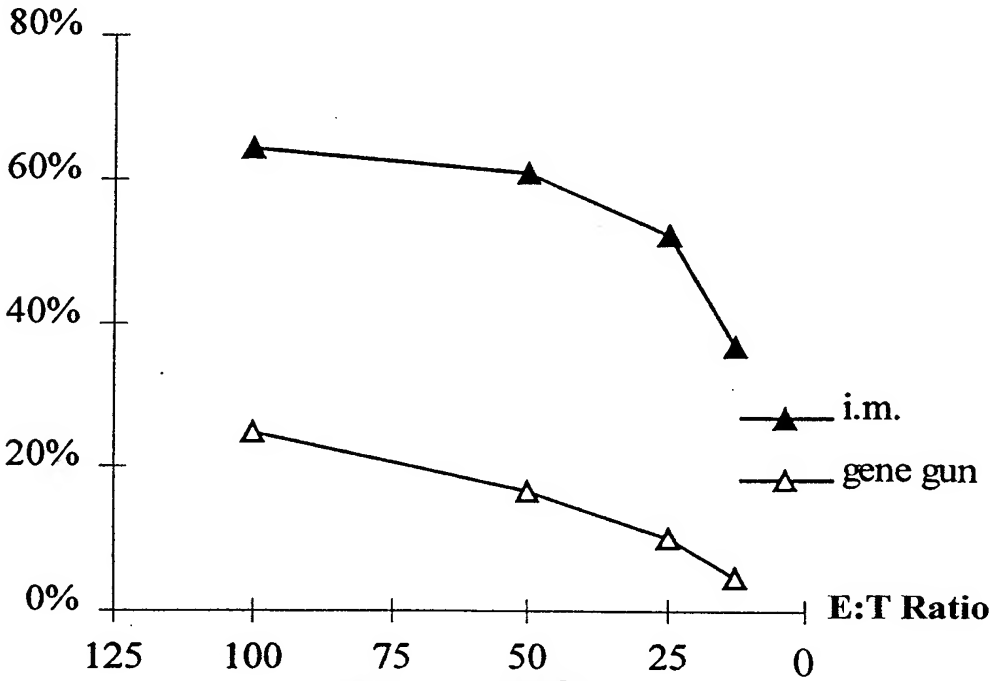

Fig. 26

		Percentage of antiserum positive to $gp120_{IIIb}$ and $gp41_{IIIb}$ in western blot assay		
Week		0	9	18
syn.$gp120_{BX08}$	gp120	0	65	90
	gp41	0	0	35
syn.$gp140_{BX08}$	gp120	0	65	100
	gp41	0	95	100
syn.$gp150_{BX08}$	gp120	0	30	41
	gp41	0	41	53
syn.$gp160_{BX08}$	gp120	0	32	50
	gp41	0	44	64
wt.$gp160_{BX08}$	gp120	0	nd	53
	gp41	0	nd	48
wt.$gp160_{BX08}$/pRev	gp120	0	nd	5
	gp41	0	nd	55

Fig. 27

Fig. 28 A

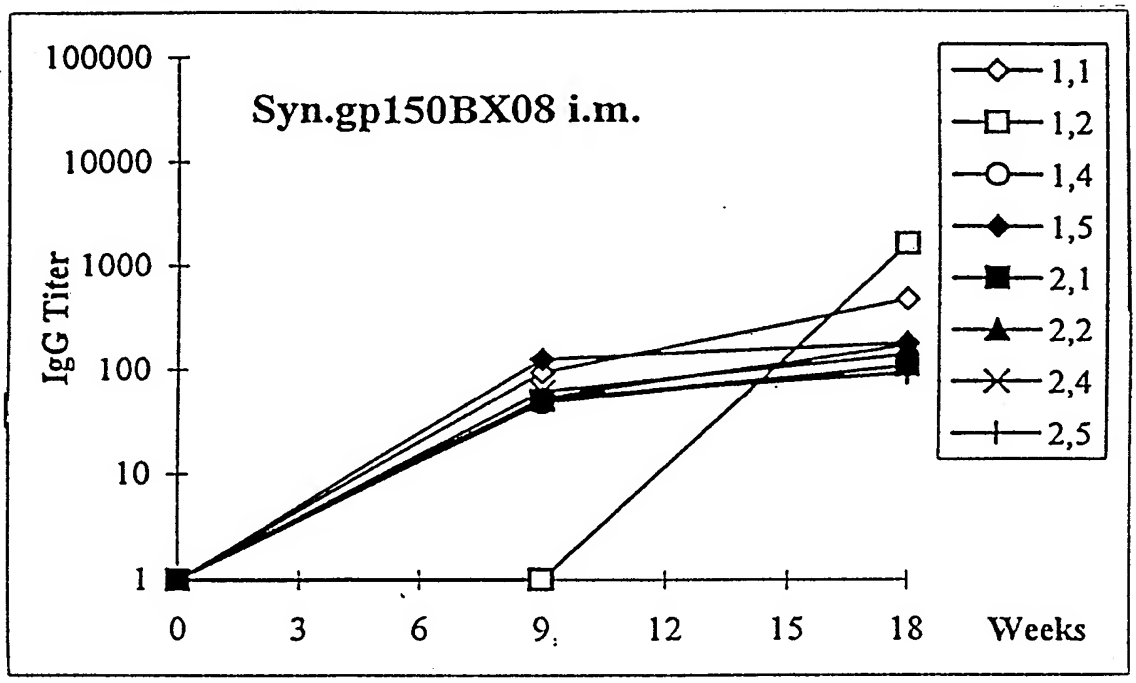

Fig. 28 B

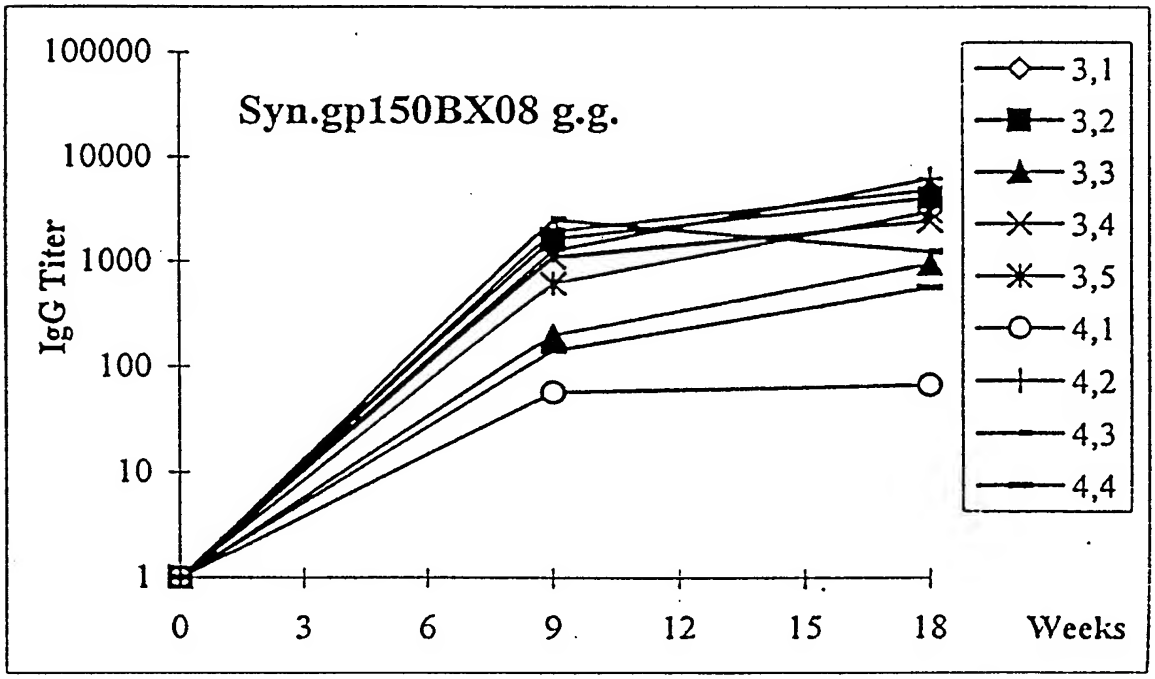

Fig. 28

Fig. 29 A

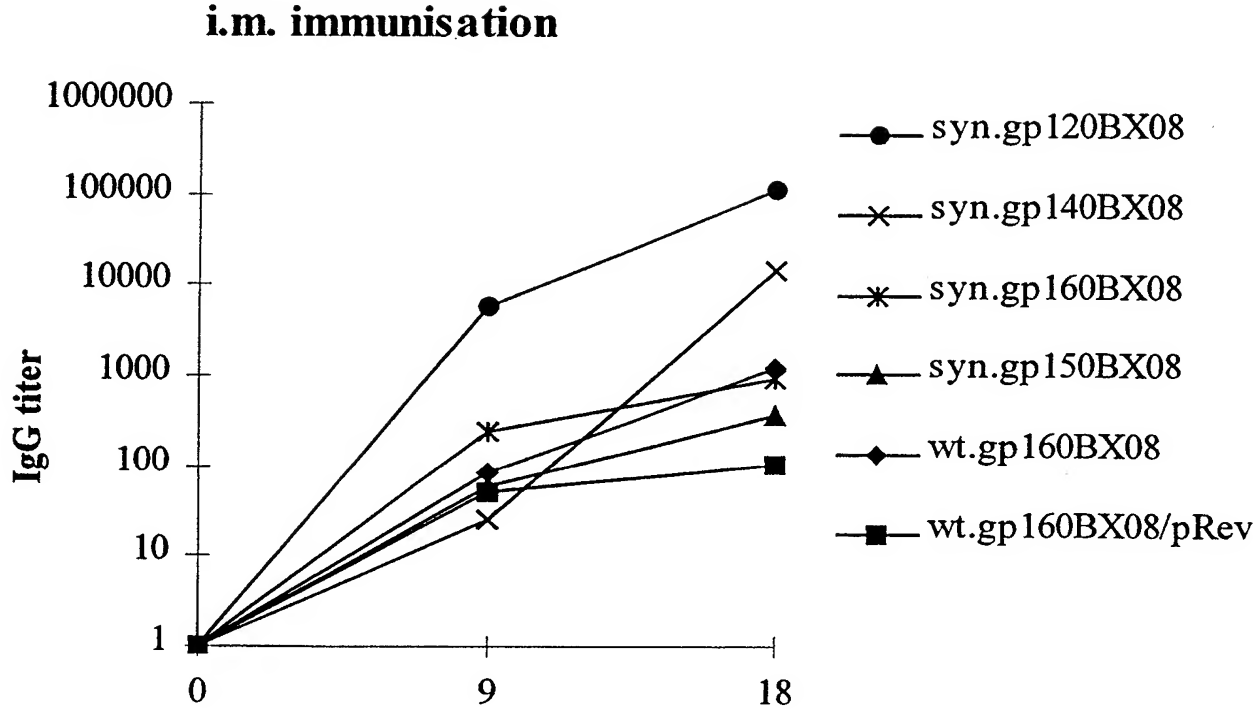

Fig. 29 B

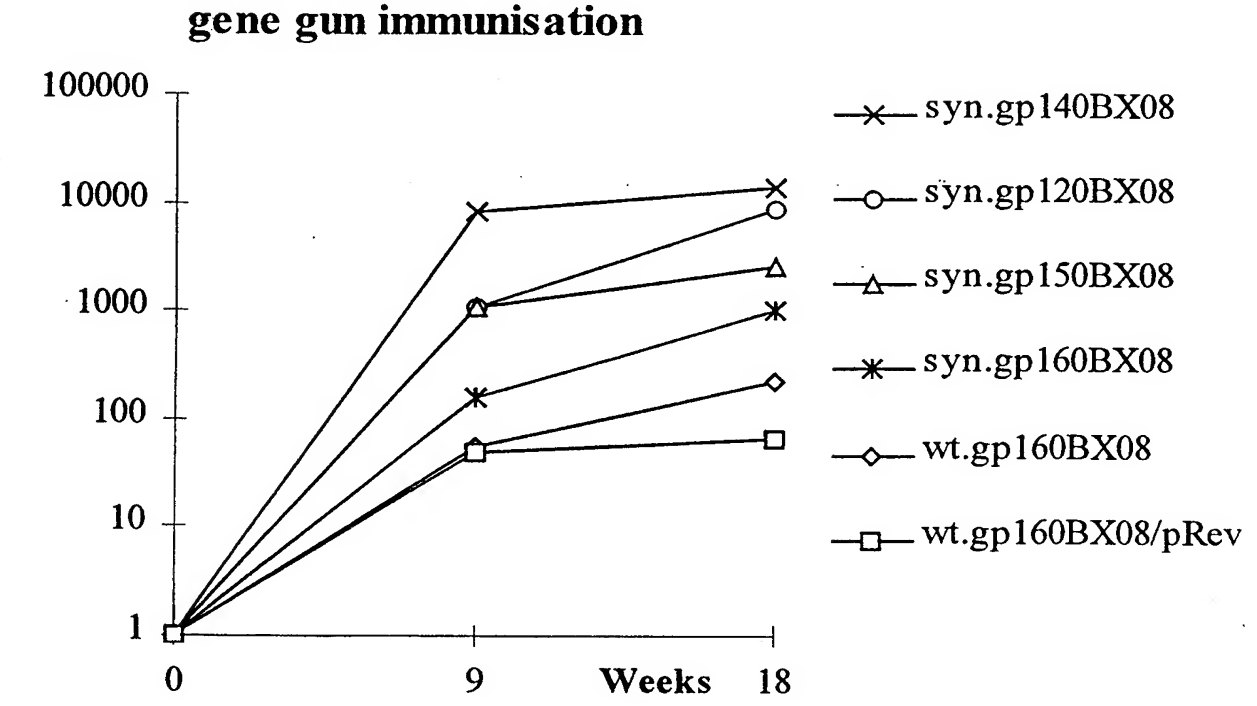

Fig. 29

SEQUENCE LISTING

<110> Statens Serum Institut

<120> Method for producing an nucleotide sequence construct with optimised codons for an HIV envelope based on a primary, clinical HIV isolate and the BX08 construct.

<130> 21924DK1

<160> 76

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 243
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(243)

<400> 1

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc       48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag       96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg      144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag      192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat      240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc                                                                 243
Ile
```

<210> 2
<211> 81
<212> PRT
<213> Artificial Sequence

<400> 2

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
```

```
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile
```

<210> 3
<211> 143
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(143)

<400> 3

```
gat atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg        48
Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu
 1               5                  10                  15

acc ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc        96
Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr
            20                  25                  30

gac acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc ag       143
Asp Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys
        35                  40                  45
```

<210> 4
<211> 47
<212> PRT
<213> Artificial Sequence

<400> 4

```
Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu
 1               5                  10                  15
Thr Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr
            20                  25                  30
Asp Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys
        35                  40                  45
```

<210> 5
<211> 132
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(132)

<400> 5

```
ctg cag ctt caa cat cag cac cag cgt gcg caa caa gat gaa gcg cga        48
Leu Gln Leu Gln His Gln His Gln Arg Ala Gln Gln Asp Glu Ala Arg
```

```
 1               5                  10                  15

gta cgc cct gtt cta cag cct gga cat cgt gcc cat cga caa cga caa        96
Val Arg Pro Val Leu Gln Pro Gly His Arg Ala His Arg Gln Arg Gln
            20                  25                  30

cac cag cta ccg cct gcg cag ctg caa cac atc gat                       132
His Gln Leu Pro Pro Ala Gln Leu Gln His Ile Asp
        35                  40


      <210> 6
      <211> 44
      <212> PRT
      <213> Artificial Sequence

      <400> 6
Leu Gln Leu Gln His Gln His Gln Arg Ala Gln Gln Asp Glu Ala Arg
 1               5                  10                  15
Val Arg Pro Val Leu Gln Pro Gly His Arg Ala His Arg Gln Arg Gln
            20                  25                  30
His Gln Leu Pro Pro Ala Gln Leu Gln His Ile Asp
        35                  40

      <210> 7
      <211> 161
      <212> DNA
      <213> Artificial Sequence

      <220>
      <221> CDS
      <222> (1)...(161)

      <400> 7
atc gat cat cac cca ggc ctg ccc caa ggt gag ctt cga gcc cat ccc        48
Ile Asp His His Pro Gly Leu Pro Gln Gly Glu Leu Arg Ala His Pro
 1               5                  10                  15

cat cca ctt ctg cgc ccc cgc cgg ctt cgc cat cct gaa gtg caa caa        96
His Pro Leu Leu Arg Pro Arg Arg Leu Arg His Pro Glu Val Gln Gln
            20                  25                  30

caa gac ctt caa cgg cac cgg ccc ctg cac caa cgt gag cac cgt gca       144
Gln Asp Leu Gln Arg His Arg Pro Leu His Gln Arg Glu His Arg Ala
        35                  40                  45

gtg cac cca cgg aat tc                                               161
Val His Pro Arg Asn
    50


      <210> 8
      <211> 53
      <212> PRT
      <213> Artificial Sequence

      <400> 8
Ile Asp His His Pro Gly Leu Pro Gln Gly Glu Leu Arg Ala His Pro
 1               5                  10                  15
```

```
His Pro Leu Leu Arg Pro Arg Arg Leu Arg His Pro Glu Val Gln Gln
            20                  25                  30
Gln Asp Leu Gln Arg His Arg Pro Leu His Gln Arg Glu His Arg Ala
        35                  40                  45
Val His Pro Arg Asn
    50
```

<210> 9
<211> 254
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(254)

<400> 9

```
gaa ttc gcc ccg tgg tga gca ccc agc tgc tgc tga acg gca gcc tgg       48
Glu Phe Ala Pro Trp  *  Ala Pro Ser Cys Cys  *  Thr Ala Ala Trp
 1               5                  10

ccg agg agg agg tgg tga tca gat ctg aga act tca cca aca acg cca       96
Pro Arg Arg Arg Trp  *  Ser Asp Leu Arg Thr Ser Pro Thr Thr Pro
 15                  20                  25

aga cca tca tcg tgc agc tga acg aga gcg tgg aga tca act gca ccc      144
Arg Pro Ser Ser Cys Ser  *  Thr Arg Ala Trp Arg Ser Thr Ala Pro
 30                  35                  40

gcc cca aca aca aca ccc gca aga gca tcc aca tcg gcc ctg gcc gcg      192
Ala Pro Thr Thr Thr Pro Ala Arg Ala Ser Thr Ser Ala Leu Ala Ala
 45                  50                  55                  60

cct tct aca cca ccg gcg aca tca tcg gcg aca tcc gcc agg ccc act      240
Pro Ser Thr Pro Pro Ala Thr Ser Ser Ala Thr Ser Ala Arg Pro Thr
                65                  70                  75

gca aca tct cta ga                                                  254
Ala Thr Ser Leu
             80
```

<210> 10
<211> 80
<212> PRT
<213> Artificial Sequence

<400> 10

```
Glu Phe Ala Pro Trp Ala Pro Ser Cys Cys Thr Ala Ala Trp Pro Arg
 1               5                  10                  15
Arg Arg Trp Ser Asp Leu Arg Thr Ser Pro Thr Thr Pro Arg Pro Ser
            20                  25                  30
Ser Cys Ser Thr Arg Ala Trp Arg Ser Thr Ala Pro Ala Pro Thr Thr
        35                  40                  45
Thr Pro Ala Arg Ala Ser Thr Ser Ala Leu Ala Ala Pro Ser Thr Pro
    50                  55                  60
Pro Ala Thr Ser Ser Ala Thr Ser Ala Arg Pro Thr Ala Thr Ser Leu
65                  70                  75                  80
```

<210> 11
<211> 92
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(92)

<400> 11

```
tct aga acc aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg        48
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 -               5                  10                  15

cgc gag aag ttc aac aac acc acc atc gtg ttc aac cag agc tc           92
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser
             20                  25                  30
```

<210> 12
<211> 30
<212> PRT
<213> Artificial Sequence

<400> 12

```
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 1               5                  10                  15
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser
            20                  25                  30
```

<210> 13
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(130)

<400> 13

```
gag ctc cgg cgg cga ccc cga gat cgt gat gca cag ctt caa ctg cgg        48
Glu Leu Arg Arg Arg Pro Arg Asp Arg Asp Ala Gln Leu Gln Leu Arg
 1               5                  10                  15

cgg cga gtt ctt cta ctg caa cac cac cca gct gtt caa cag cac ctg        96
Arg Arg Val Leu Leu Leu Gln His His Pro Ala Val Gln Gln His Leu
             20                  25                  30

gaa cga gac caa cag cga ggg caa cat cac tag t                        130
Glu Arg Asp Gln Gln Arg Gly Gln His His  *
         35                  40
```

<210> 14
<211> 42
<212> PRT
<213> Artificial Sequence

<400> 14

```
Glu Leu Arg Arg Arg Pro Arg Asp Arg Asp Ala Gln Leu Gln Leu Arg
 1               5                  10                  15
Arg Arg Val Leu Leu Leu Gln His His Pro Ala Val Gln Gln His Leu
            20                  25                  30
Glu Arg Asp Gln Gln Arg Gly Gln His His
        35                  40
```

<210> 15
<211> 164
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(164)

<400> 15

```
act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag atc atc aac       48
Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn
 1               5                  10                  15

atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc atc ggc ggc       96
Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Gly Gly
            20                  25                  30

cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg acc cgc gac      144
Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp
        35                  40                  45

ggc ggc agc gac aac tcg ag                                           164
Gly Gly Ser Asp Asn Ser
    50
```

<210> 16
<211> 54
<212> PRT
<213> Artificial Sequence

<400> 16

```
Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asn
 1               5                  10                  15
Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile Gly Gly
            20                  25                  30
Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp
        35                  40                  45
Gly Gly Ser Asp Asn Ser
    50
```

<210> 17
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(200)

<400> 17

```
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg        48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga        96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg       144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45

~~cga~~ gaa gcg cgc cgt ggg cat cgg ~~cgc tat~~ gtt cct cgg ctt cct ggg  192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60

cgc tgc ag                                                          200
Arg Cys
 65
```

<210> 18
<211> 66
<212> PRT
<213> Artificial Sequence

<400> 18

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys
65
```

<210> 19
<211> 212
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(212)

<400> 19

```
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg        48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga        96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg       144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
```

```
cga gaa gcg cgc cta ggg cat cgg cgc tat gtt cct cgg ctt cct ggg      192
Arg Glu Ala Arg Leu Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
     50                  55                  60

cgc tgc agc ccg ggg gat cc                                          212
Arg Cys Ser Pro Gly Asp
 65                  70
```

<210> 20
<211> 70
<212> PRT
<213> Artificial Sequence

<400> 20

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Ala Arg Leu Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys Ser Pro Gly Asp
65                  70
```

<210> 21
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(200)

<400> 21

```
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg       48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga       96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg      144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45

cga gaa gag cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg      192
Arg Glu Glu Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
     50                  55                  60

cgc tgc ag                                                          200
Arg Cys
 65
```

<210> 22

<211> 66
<212> PRT
<213> Artificial Sequence

<400> 22

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Glu Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys
65
```

<210> 23
<211> 178
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(178)

<400> 23

```
ctg cag gca gca cca tgg gcg ccg cca gcc tga ccc tga ccg tgc agg       48
Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala  *  Pro  *  Pro Cys Arg
 1               5                  10

ccc gcc agc tgc tga gcg gca tcg tgc agc agc aga aca acc tgc tgc       96
Pro Ala Ser Cys  *  Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys
 15                  20                  25

gcg cca tcg agg ccc agc agc acc tgc tcc agc tga ccg tgt ggg gca      144
Ala Pro Ser Arg Pro Ser Ser Thr Cys Ser Ser  *  Pro Cys Gly Ala
 30                  35                  40

tca agc agc tcc agg ccc gcg tgc tgg ctc tag a                       178
Ser Ser Ser Ser Arg Pro Ala Cys Trp Leu  *
 45                  50
```

<210> 24
<211> 54
<212> PRT
<213> Artificial Sequence

<400> 24

```
Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala Pro Pro Cys Arg Pro Ala
 1               5                  10                  15
Ser Cys Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys Ala Pro Ser
            20                  25                  30
Arg Pro Ser Ser Thr Cys Ser Ser Pro Cys Gly Ala Ser Ser Ser Ser
        35                  40                  45
Arg Pro Ala Cys Trp Leu
    50
```

<210> 25

<211> 178
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(178)

<400> 25

```
ctg cag gca gca cca tgg gcg ccg cca gcc tga ccc tga ccg tgc agg        48
Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala  *  Pro  *  Pro Cys Arg
 1               5                  10

ccc gcc agc tgc tga gcg gca tcg tgc agc agc aga aca acc tgc tgc        96
Pro Ala Ser Cys  *  Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys
 15                  20                  25

gcg cca tcg agg ccc agc agc acc tgc tcc agc tga ccg tgt ggg gca       144
Ala Pro Ser Arg Pro Ser Ser Thr Cys Ser Ser  *  Pro Cys Gly Ala
 30                  35                  40

tca agc agt gct gcg gcc gcg tgc tgg ctc tag a                         178
Ser Ser Ser Ala Ala Ala Ala Cys Trp Leu  *
 45                  50
```

<210> 26
<211> 54
<212> PRT
<213> Artificial Sequence

<400> 26

```
Leu Gln Ala Ala Pro Trp Ala Pro Pro Ala Pro Pro Cys Arg Pro Ala
 1               5                  10                  15
Ser Cys Ala Ala Ser Cys Ser Ser Arg Thr Thr Cys Cys Ala Pro Ser
            20                  25                  30
Arg Pro Ser Ser Thr Cys Ser Ser Pro Cys Gly Ala Ser Ser Ser Ala
        35                  40                  45
Ala Ala Ala Cys Trp Leu
    50
```

<210> 27
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(77)

<400> 27

```
tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg        48
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15

ctc cgg caa gct gat ctg cac cac ggc cg                                77
Leu Arg Gln Ala Asp Leu His His Gly
            20                  25
```

<210> 28
<211> 25
<212> PRT
<213> Artificial Sequence

<400> 28
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
1 5 10 15
Leu Arg Gln Ala Asp Leu His His Gly
20 25

<210> 29
<211> 190
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(190)

<400> 29
cgg ccg tgc cct gga acg cca gct gga gca aca aga acc tga gcc aga 48
Arg Pro Cys Pro Gly Thr Pro Ala Gly Ala Thr Arg Thr * Ala Arg
1 5 10 15

ttt ggg aca aca tga cct gga tgg agt ggg agc gcg aga tca gca act 96
Phe Gly Thr Thr * Pro Gly Trp Ser Gly Ser Ala Arg Ser Ala Thr
20 25 30

aca ccg aga tca tct aca gcc tga tcg agg aga gcc aga acc agc agg 144
Thr Pro Arg Ser Ser Thr Ala * Ser Arg Arg Ala Arg Thr Ser Arg
35 40 45

aga aga acg agc tgg acc tgc tcc agc tgg aca agt ggg caa gct t 190
Arg Arg Thr Ser Trp Thr Cys Ser Ser Trp Thr Ser Gly Gln Ala
50 55 60

<210> 30
<211> 60
<212> PRT
<213> Artificial Sequence

<400> 30
Arg Pro Cys Pro Gly Thr Pro Ala Gly Ala Thr Arg Thr Ala Arg Phe
1 5 10 15
Gly Thr Thr Pro Gly Trp Ser Gly Ser Ala Arg Ser Ala Thr Thr Pro
20 25 30
Arg Ser Ser Thr Ala Ser Arg Arg Ala Arg Thr Ser Arg Arg Arg Thr
35 40 45
Ser Trp Thr Cys Ser Ser Trp Thr Ser Gly Gln Ala
50 55 60

<210> 31
<211> 177
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(177)

<400> 31
```
aag ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa        48
Lys Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln
 1               5                  10                  15

gat ttt cat cat gat cgt ggg cgg cct gat cgg cct gcg cat cgt gtt        96
Asp Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val
            20                  25                  30

cac cgt gct gag cat cgt gaa ccg cgt gcg cca ggg cta cag ccc cct       144
His Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro
        35                  40                  45

gag ctt cca gac ccg cct gcc cgt gcc ccg cgg                           177
Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg
    50                  55
```

<210> 32
<211> 59
<212> PRT
<213> Artificial Sequence

<400> 32
```
Lys Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln
 1               5                  10                  15
Asp Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val
            20                  25                  30
His Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro
        35                  40                  45
Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg
    50                  55
```

<210> 33
<211> 140
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(140)

<400> 33
```
ccg cgg ccc cga ccg ccc cga ggg cat cga gga gga ggg cgg cga gcg        48
Pro Arg Pro Arg Pro Pro Arg Gly His Arg Gly Gly Gly Arg Arg Ala
 1               5                  10                  15

cga ccg cga ccg cag cac ccg cct ggt gac cgg ctt cct gcc cct gat        96
Arg Pro Arg Pro Gln His Pro Pro Gly Asp Arg Leu Pro Ala Pro Asp
            20                  25                  30

ctg gga cga cct gcg cag cct gtt cct gtt cag cta cca tcg at            140
Leu Gly Arg Pro Ala Gln Pro Val Pro Val Gln Leu Pro Ser
        35                  40                  45
```

<210> 34
<211> 46
<212> PRT
<213> Artificial Sequence

<400> 34
Pro Arg Pro Arg Pro Pro Arg Gly His Arg Gly Gly Gly Arg Arg Ala
1 5 10 15
Arg Pro Arg Pro Gln His Pro Pro Gly Asp Arg Leu Pro Ala Pro Asp
20 25 30
Leu Gly Arg Pro Ala Gln Pro Val Pro Val Gln Leu Pro Ser
35 40 45

<210> 35
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(129)

<400> 35
atc gat tgc gcg acc tgc tgc tga tcg tgg ccc gca tcg tgg agc tgc 48
Ile Asp Cys Ala Thr Cys Cys * Ser Trp Pro Ala Ser Trp Ser Cys
1 5 10 15

tgg gcc ggc gcg gct ggg aga tcc tga agt act ggt gga acc tgc tcc 96
Trp Ala Gly Ala Ala Gly Arg Ser * Ser Thr Gly Gly Thr Cys Ser
20 25 30

agt act gga gcc agg agc tga aga act ctg cag 129
Ser Thr Gly Ala Arg Ser * Arg Thr Leu Gln
35 40

<210> 36
<211> 40
<212> PRT
<213> Artificial Sequence

<400> 36
Ile Asp Cys Ala Thr Cys Cys Ser Trp Pro Ala Ser Trp Ser Cys Trp
1 5 10 15
Ala Gly Ala Ala Gly Arg Ser Ser Thr Gly Gly Thr Cys Ser Ser Thr
20 25 30
Gly Ala Arg Ser Arg Thr Leu Gln
35 40

<210> 37
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(114)

<400> 37
ctg cag tga gcc tgc tga acg cca ccg cca tcg ccg tgg ccg agg gca 48
Leu Gln * Ala Cys * Thr Pro Pro Pro Ser Pro Trp Pro Arg Ala
1 5 10

ccg acc gcg tga tcg agg tgg tgc agc gca tct ggc gcg gca tcc tgc 96
Pro Thr Ala * Ser Arg Trp Cys Ser Ala Ser Gly Ala Ala Ser Cys
15 20 25

aca tcc cca ccc gaa ttc 114
Thr Ser Pro Pro Glu Phe
30 35

<210> 38
<211> 35
<212> PRT
<213> Artificial Sequence

<400> 38
Leu Gln Ala Cys Thr Pro Pro Pro Ser Pro Trp Pro Arg Ala Pro Thr
1 5 10 15
Ala Ser Arg Trp Cys Ser Ala Ser Gly Ala Ala Ser Cys Thr Ser Pro
20 25 30
Pro Glu Phe
35

<210> 39
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(41)

<400> 39
gaa ttc gcc agg gct tcg agc gcg ccc tgc tgt aag gat cc 41
Glu Phe Ala Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
1 5 10

<210> 40
<211> 13
<212> PRT
<213> Artificial Sequence

<400> 40
Glu Phe Ala Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
1 5 10

<210> 41
<211> 506
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(506)

<400> 41

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc       48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag       96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg      144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag      192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat      240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc      288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac      336
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc      384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg      432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac      480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg at                                   506
Arg Leu Arg Ser Cys Asn Thr Ser
                165
```

<210> 42
<211> 168
<212> PRT
<213> Artificial Sequence

<400> 42

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
```

```
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser
                165
```

<210> 43
<211> 374
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(374)

<400> 43

```
tct aga acc aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg        48
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 1               5                  10                  15

cgc gag aag ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc        96
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly
            20                  25                  30

ggc gac ccc gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc       144
Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
        35                  40                  45

ttc tac tgc aac acc acc cag ctg ttc aac agc acc tgg aac gag acc       192
Phe Tyr Cys Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr
    50                  55                  60

aac agc gag ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc       240
Asn Ser Glu Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg
65                  70                  75                  80

atc aag cag atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac       288
Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
                85                  90                  95

gcc ccc ccc atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc       336
Ala Pro Pro Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly
            100                 105                 110

ctg ctg ctg acc cgc gac ggc ggc agc gac aac tcg ag                    374
Leu Leu Leu Thr Arg Asp Gly Gly Ser Asp Asn Ser
        115                 120
```

<210> 44
<211> 124
<212> PRT
<213> Artificial Sequence

<400> 44

```
Ser Arg Thr Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu
 1               5                  10                  15
Arg Glu Lys Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly
            20                  25                  30
Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe
        35                  40                  45
Phe Tyr Cys Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr
    50                  55                  60
Asn Ser Glu Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg
65                  70                  75                  80
Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
                85                  90                  95
Ala Pro Pro Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly
            100                 105                 110
Leu Leu Leu Thr Arg Asp Gly Gly Ser Asp Asn Ser
        115                 120
```

<210> 45
<211> 1277
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(1277)

<400> 45

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc       48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag       96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg      144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag      192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat      240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc      288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac      336
```

```
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc      384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg      432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac      480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag      528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc      576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc      624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205

acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg      672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg      720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240

atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
```

```
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc      1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag      1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag      1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc      1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg      1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg ag                               1277
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 46
<211> 425
<212> PRT
<213> Artificial Sequence

<400> 46

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
```

```
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 47
<211> 1277
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(1277)

<400> 47

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc        48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag        96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg       144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag       192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat       240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc       288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
```

```
                    85                  90                  95

ccc ctg tgc gtg acc ctg caa tgc acc aag ctg aag cag agc acc gac      336
Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp
            100                 105                 110

acc cag aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc      384
Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg      432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac      480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag      528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc      576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc      624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205

acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg      672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg      720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240

atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
```

```
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc      1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag      1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag      1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc      1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg      1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg ag                               1277
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 48
<211> 425
<212> PRT
<213> Artificial Sequence

<400> 48

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp
            100                 105                 110
Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
```

```
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 49
<211> 144
<212> PRT
<213> Artificial Sequence

<400> 49

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc      48
gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag      96
gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg     144
ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag     192
aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat     240
atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc     288
ccc ctg tgc gtg acc ctg caa tgc acc aag ctg aag cag agc acc gac     336
acc cag aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc     384
cag atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg     432
ttc tac agc ctg gac atc gtg ccc atc gac aac gac cag acc agc tac     480
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag     528
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc     576
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc     624
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg     672
agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg     720
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag     768
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc     816
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc     864
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc     912
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag     960
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc    1008
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc    1056
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag    1104
```

```
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag     1152
atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc     1200
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg     1248
acc cgc gac ggc ggc agc gac aac tcg ag                              1277
```

<210> 50
<211> 425
<212> PRT
<213> Artificial Sequence

<400> 50

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Gln Cys Thr Lys Leu Lys Gln Ser Thr Asp
            100                 105                 110
Thr Gln Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Gln Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Gln Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
```

```
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 51
<211> 144
<212> PRT
<213> Artificial Sequence

<400> 51

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc        48
gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag        96
gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg       144
ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag       192
aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat       240
atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc       288
ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac       336
acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc       384
cag atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg       432
ttc tac agc ctg gac atc gtg ccc atc gac aac gac cag acc agc tac       480
cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag       528
gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc       576
gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc       624
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg       672
agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg       720
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag       768
ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc       816
cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc       864
gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc       912
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag       960
ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc      1008
gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc      1056
aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag      1104
ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag      1152
atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc      1200
atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg      1248
acc cgc gac ggc ggc agc gac aac tcg ag                              1277
```

<210> 52
<211> 425
<212> PRT
<213> Artificial Sequence

<400> 52

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
```

```
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Gln Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Gln Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser
            420                 425
```

<210> 53
<211> 432
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(432)

<400> 53

```
tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg        48
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15

ctc cgg caa gct gat ctg cac cac ggc cgt gcc ctg gaa cgc cag ctg        96
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
```

```
                20                  25                  30

gag caa caa gaa cct gag cca gat ttg gga caa cat gac ctg gat gga      144
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
         35                  40                  45

gtg gga gcg cga gat cag caa cta cac cga gat cat cta cag cct gat      192
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
     50                  55                  60

cga gga gag cca gaa cca gca gga gaa gaa cga gct gga cct gct cca      240
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
 65                  70                  75                  80

gct gga caa gtg ggc aag ctt gtg gaa ctg gtt caa cat cac caa ctg      288
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu
                 85                  90                  95

gct gtg gta cat caa gat ttt cat cat gat cgt ggg cgg cct gat cgg      336
Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg
            100                 105                 110

cct gcg cat cgt gtt cac cgt gct gag cat cgt gaa ccg cgt gcg cca      384
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro
        115                 120                 125

ggg cta cag ccc cct gag ctt cca gac ccg cct gcc cgt gcc ccg cgg      432
Gly Leu Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg
    130                 135                 140
```

<210> 54
<211> 144
<212> PRT
<213> Artificial Sequence

<400> 54

```
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
            20                  25                  30
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
        35                  40                  45
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
    50                  55                  60
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
65                  70                  75                  80
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu
                85                  90                  95
Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg
            100                 105                 110
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro
        115                 120                 125
Gly Met Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
    130                 135                 140
```

<210> 55
<211> 434
<212> DNA

<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(434)

<400> 55

```
tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg        48
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15

ctc cgg caa gct gat ctg cac cac ggc cgt gcc ctg gaa cgc cag ctg        96
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
            20                  25                  30

gag caa caa gaa cct gag cca gat ttg gga caa cat gac ctg gat gga       144
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
        35                  40                  45

gtg gga gcg cga gat cag caa cta cac cga gat cat cta cag cct gat       192
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
    50                  55                  60

cga gga gag cca gaa cca gca gga gaa gaa cga gct gga cct gct cca       240
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
65                  70                  75                  80

gct gga caa gtg ggc aag ctt gtg gaa ctg gtt caa cat cac caa ctg       288
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu
                85                  90                  95

gct gtg gta cat caa gat ttt cat cat gat cgt ggg cgg cct gat cgg       336
Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg
            100                 105                 110

cct gcg cat cgt gtt cac cgt gct gag cat cgt gaa ccg cgt gcg cca       384
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro
        115                 120                 125

ggg atg cag ccc cct gag ctt cca gac ccg cct gcc cgt gtg acg gat       432
Gly Met Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
    130                 135                 140

cc                                                                   434
```

<210> 56
<211> 144
<212> PRT
<213> Artificial Sequence

<400> 56

```
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
            20                  25                  30
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
        35                  40                  45
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
    50                  55                  60
```

```
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
65                  70                  75                  80
Ala Gly Gln Val Gly Lys Leu Val Glu Leu Val Gln His His Gln Leu
                85                  90                  95
Ala Val Val His Gln Asp Phe His His Asp Arg Gly Arg Pro Asp Arg
            100                 105                 110
Pro Ala His Arg Val His Arg Ala Glu His Arg Glu Pro Arg Ala Pro
        115                 120                 125
Gly Met Gln Pro Pro Glu Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
    130                 135                 140
```

<210> 57
<211> 281
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(281)

<400> 57

```
tct aga gcg cta cct cca gga cca gcg ctt cct ggg cat gtg ggg ctg        48
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15

ctc cgg caa gct gat ctg cac cac ggc cgt gcc ctg gaa cgc cag ctg        96
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
             20                  25                  30

gag caa caa gaa cct gag cca gat ttg gga caa cat gac ctg gat gga       144
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
         35                  40                  45

gtg gga gcg cga gat cag caa cta cac cga gat cat cta cag cct gat       192
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
     50                  55                  60

cga gga gag cca gaa cca gca gga gaa gaa cga gct gga cct gct cca       240
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
 65                  70                  75                  80

gct gga caa gtg ggc aag ctt gtg tga ctg att gag gat cc              281
Ala Gly Gln Val Gly Lys Leu Val  *  Leu Ile Glu Asp
                85                  90
```

<210> 58
<211> 92
<212> PRT
<213> Artificial Sequence

<400> 58

```
Ser Arg Ala Leu Pro Pro Gly Pro Ala Leu Pro Gly His Val Gly Leu
 1               5                  10                  15
Leu Arg Gln Ala Asp Leu His His Gly Arg Ala Leu Glu Arg Gln Leu
             20                  25                  30
Glu Gln Gln Glu Pro Glu Pro Asp Leu Gly Gln His Asp Leu Asp Gly
         35                  40                  45
Val Gly Ala Arg Asp Gln Gln Leu His Arg Asp His Leu Gln Pro Asp
```

```
    50                  55                  60
Arg Gly Glu Pro Glu Pro Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro
65                  70                  75                  80
Ala Gly Gln Val Gly Lys Leu Val Leu Ile Glu Asp
                85                  90
```

<210> 59
<211> 272
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(272)

<400> 59

```
atc gat tgc gcg acc tgc tgc tga tcg tgg ccc gca tcg tgg agc tgc        48
Ile Asp Cys Ala Thr Cys Cys  *  Ser Trp Pro Ala Ser Trp Ser Cys
 1               5                  10                  15

tgg gcc ggc gcg gct ggg aga tcc tga agt act ggt gga acc tgc tcc        96
Trp Ala Gly Ala Ala Gly Arg Ser  *  Ser Thr Gly Gly Thr Cys Ser
            20                  25                  30

agt act gga gcc agg agc tga aga act ctg cag tga gcc tgc tga acg       144
Ser Thr Gly Ala Arg Ser  *  Arg Thr Leu Gln  *  Ala Cys  *  Thr
        35                  40

cca ccg cca tcg ccg tgg ccg agg gca ccg acc gcg tga tcg agg tgg       192
Pro Pro Pro Ser Pro Trp Pro Arg Ala Pro Thr Ala  *  Ser Arg Trp
    45                  50                  55

tgc agc gca tct ggc gcg gca tcc tgc aca tcc cca ccc gaa ttc gcc       240
Cys Ser Ala Ser Gly Ala Ala Ser Cys Thr Ser Pro Pro Glu Phe Ala
    60                  65                  70

agg gct tcg agc gcg ccc tgc tgt aag gat cc                           272
Arg Ala Ser Ser Ala Pro Cys Cys Lys Asp
75                  80
```

<210> 60
<211> 84
<212> PRT
<213> Artificial Sequence

<400> 60

```
Ile Asp Cys Ala Thr Cys Cys Ser Trp Pro Ala Ser Trp Ser Cys Trp
 1               5                  10                  15
Ala Gly Ala Ala Gly Arg Ser Ser Thr Gly Gly Thr Cys Ser Ser Thr
            20                  25                  30
Gly Ala Arg Ser Arg Thr Leu Gln Ala Cys Thr Pro Pro Pro Ser Pro
        35                  40                  45
Trp Pro Arg Ala Pro Thr Ala Ser Arg Trp Cys Ser Ala Ser Gly Ala
    50                  55                  60
Ala Ser Cys Thr Ser Pro Pro Glu Phe Ala Arg Ala Ser Ser Ala Pro
65                  70                  75                  80
Cys Cys Lys Asp
```

<210> 61
<211> 798
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(798)

<400> 61

```
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg        48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga        96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg       144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45

cga gaa gcg cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg       192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60

cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca       240
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80

ggc ccg cca gct gct gag cgg cat cgt gca gca gca gaa caa cct gct       288
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95

gcg cgc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg       336
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110

cat caa gca gct cca ggc ccg cgt gct ggc tct aga gcg cta cct cca       384
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125

gga cca gcg ctt cct ggg cat gtg ggg ctg ctc cgg caa gct gat ctg       432
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140

cac cac ggc cgt gcc ctg gaa cgc cag ctg gag caa caa gaa cct gag       480
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160

cca gat ttg gga caa cat gac ctg gat gga gtg gga gcg cga gat cag       528
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175

caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca       576
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
            180                 185                 190
```

```
gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag      624
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205

ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa gat      672
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp
    210                 215                 220

ttt cat cat gat cgt ggg cgg cct gat cgg cct gcg cat cgt gtt cac      720
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His
225                 230                 235                 240

cgt gct gag cat cgt gaa ccg cgt gcg cca ggg cta cag ccc cct gag      768
Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro Glu
                245                 250                 255

ctt cca gac ccg cct gcc cgt gcc ccg cgg                             798
Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg
            260                 265
```

<210> 62
<211> 266
<212> PRT
<213> Artificial Sequence

<400> 62

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
            180                 185                 190
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp
    210                 215                 220
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His
225                 230                 235                 240
Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Leu Gln Pro Pro Glu
                245                 250                 255
```

```
Leu Pro Asp Pro Pro Ala Arg Ala Pro Arg
            260                 265

      <210> 63
      <211> 800
      <212> DNA
      <213> Artificial Sequence

      <220>
      <221> CDS
      <222> (1)...(800)

      <400> 63
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg       48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga       96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg      144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45

cga gaa gcg cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg      192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60

cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca      240
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80

ggc ccg cca gct gct gag cgg cat cgt gca gca gca gaa caa cct gct      288
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95

gcg cgc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg      336
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110

cat caa gca gct cca ggc ccg cgt gct ggc tct aga gcg cta cct cca      384
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125

gga cca gcg ctt cct ggg cat gtg ggg ctg ctc cgg caa gct gat ctg      432
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140

cac cac ggc cgt gcc ctg gaa cgc cag ctg gag caa caa gaa cct gag      480
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160

cca gat ttg gga caa cat gac ctg gat gga gtg gga gcg cga gat cag      528
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175

caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca      576
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
```

```
                180                 185                 190

gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag      624
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205

ctt gtg gaa ctg gtt caa cat cac caa ctg gct gtg gta cat caa gat      672
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp
    210                 215                 220

ttt cat cat gat cgt ggg cgg cct gat cgg cct gcg cat cgt gtt cac      720
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His
225                 230                 235                 240

cgt gct gag cat cgt gaa ccg cgt gcg cca ggg atg cag ccc cct gag      768
Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Met Gln Pro Pro Glu
                245                 250                 255

ctt cca gac ccg cct gcc cgt gtg acg gat cc                          800
Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
            260                 265
```

<210> 64
<211> 266
<212> PRT
<213> Artificial Sequence

<400> 64

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
            180                 185                 190
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205
Leu Val Glu Leu Val Gln His His Gln Leu Ala Val Val His Gln Asp
    210                 215                 220
Phe His His Asp Arg Gly Arg Pro Asp Arg Pro Ala His Arg Val His
225                 230                 235                 240
```

```
Arg Ala Glu His Arg Glu Pro Arg Ala Pro Gly Met Gln Pro Pro Glu
                245                 250                 255
Leu Pro Asp Pro Pro Ala Arg Val Thr Asp
            260                 265
```

<210> 65
<211> 647
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(647)

<400> 65

```
ctc gag cag cgg caa gga gat ttt ccg ccc cgg cgg cgg cga cat gcg        48
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15

cga caa ctg gcg cag cga gct gta caa gta caa ggt ggt gaa gat cga        96
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30

gcc cct ggg cat cgc ccc cac caa ggc caa gcg ccg cgt ggt gca gcg       144
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45

cga gaa gcg cgc cgt ggg cat cgg cgc tat gtt cct cgg ctt cct ggg       192
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60

cgc tgc agg cag cac cat ggg cgc cgc cag cct gac cct gac cgt gca       240
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80

ggc ccg cca gct gct gag cgg cat cgt gca gca gca gaa caa cct gct       288
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95

gcg cgc cat cga ggc cca gca gca cct gct cca gct gac cgt gtg ggg       336
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110

cat caa gca gct cca ggc ccg cgt gct ggc tct aga gcg cta cct cca       384
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125

gga cca gcg ctt cct ggg cat gtg ggg ctg ctc cgg caa gct gat ctg       432
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140

cac cac ggc cgt gcc ctg gaa cgc cag ctg gag caa caa gaa cct gag       480
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160

cca gat ttg gga caa cat gac ctg gat gga gtg gga gcg cga gat cag       528
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175
```

```
caa cta cac cga gat cat cta cag cct gat cga gga gag cca gaa cca      576
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
            180                 185                 190

gca gga gaa gaa cga gct gga cct gct cca gct gga caa gtg ggc aag      624
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205

ctt gtg tga ctg att gag gat cc                                      647
Leu Val  *  Leu Ile Glu Asp
    210
```

<210> 66
<211> 214
<212> PRT
<213> Artificial Sequence

<400> 66

```
Leu Glu Gln Arg Gln Gly Asp Phe Pro Pro Arg Arg Arg Arg His Ala
 1               5                  10                  15
Arg Gln Leu Ala Gln Arg Ala Val Gln Val Gln Gly Gly Glu Asp Arg
            20                  25                  30
Ala Pro Gly His Arg Pro His Gln Gly Gln Ala Pro Arg Gly Ala Ala
        35                  40                  45
Arg Glu Ala Arg Arg Gly His Arg Arg Tyr Val Pro Arg Leu Pro Gly
    50                  55                  60
Arg Cys Arg Gln His His Gly Arg Arg Gln Pro Asp Pro Asp Arg Ala
65                  70                  75                  80
Gly Pro Pro Ala Ala Glu Arg His Arg Ala Ala Ala Glu Gln Pro Ala
                85                  90                  95
Ala Arg His Arg Gly Pro Ala Ala Pro Ala Pro Ala Asp Arg Val Gly
            100                 105                 110
His Gln Ala Ala Pro Gly Pro Arg Ala Gly Ser Arg Ala Leu Pro Pro
        115                 120                 125
Gly Pro Ala Leu Pro Gly His Val Gly Leu Leu Arg Gln Ala Asp Leu
    130                 135                 140
His His Gly Arg Ala Leu Glu Arg Gln Leu Glu Gln Gln Glu Pro Glu
145                 150                 155                 160
Pro Asp Leu Gly Gln His Asp Leu Asp Gly Val Gly Ala Arg Asp Gln
                165                 170                 175
Gln Leu His Arg Asp His Leu Gln Pro Asp Arg Gly Glu Pro Glu Pro
            180                 185                 190
Ala Gly Glu Glu Arg Ala Gly Pro Ala Pro Ala Gly Gln Val Gly Lys
        195                 200                 205
Leu Val Leu Ile Glu Asp
    210
```

<210> 67
<211> 1918
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(1918)

<400> 67

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc       48
```

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag      96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg     144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag     192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat     240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc     288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac     336
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc     384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg     432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac     480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag     528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc     576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc     624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205

acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg     672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg     720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
```

```
atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

~~gac~~ atc atc ggc gac atc cgc cag ~~gcc~~ cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc     1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag     1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag     1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc     1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg     1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc     1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430

cgc ccc ggc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac     1344
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445

aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag     1392
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460

gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtg ggc atc ggc     1440
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480
```

```
gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc     1488
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495

gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc     1536
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510

gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac     1584
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525

ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtg     1632
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540

ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg     1680
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560

ggc tgc tcc ggc aag ctg atc tgc acc acg gcc gtg ccc tgg aac gcc     1728
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575

agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg     1776
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590

atg gag tgg gag cgc gag atc agc aac tac acc gag atc atc tac agc     1824
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605

ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg     1872
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620

ctc cag ctg gac aag tgg gca agc ttg tgt gac tga ttg agg atc c       1918
Leu Gln Leu Asp Lys Trp Ala Ser Leu Cys Asp  *  Leu Arg Ile
625                 630                 635
```

<210> 68
<211> 638
<212> PRT
<213> Artificial Sequence

<400> 68

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Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
```

```
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560
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Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620
Leu Gln Leu Asp Lys Trp Ala Ser Leu Cys Asp Leu Arg Ile
625                 630                 635

      <210> 69
      <211> 2071
      <212> DNA
      <213> Artificial Sequence

      <220>
      <221> CDS
      <222> (1)...(2071)

      <400> 69
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc        48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag        96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg       144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag       192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat       240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc       288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac       336
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc       384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg       432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac       480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
```

```
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag      528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc      576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc      624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205

acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg      672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg      720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240

atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc     1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag     1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag     1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc     1200
```

```
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg      1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc      1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430

cgc ccc ggc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac      1344
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445

aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag      1392
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460

gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtg ggc atc ggc      1440
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480

gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc      1488
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495

gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc      1536
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510

gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac      1584
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525

ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtg      1632
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540

ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg      1680
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560

ggc tgc tcc ggc aag ctg atc tgc acc acg gcc gtg ccc tgg aac gcc      1728
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575

agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg      1776
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590

atg gag tgg gag cgc gag atc agc aac tac acc gag atc atc tac agc      1824
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605

ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg      1872
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620
```

```
ctc cag ctg gac aag tgg gca agc ttg tgg aac tgg ttc aac atc acc     1920
Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
625                 630                 635                 640

aac tgg ctg tgg tac atc aag att ttc atc atg atc gtg ggc ggc ctg     1968
Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
                645                 650                 655

atc ggc ctg cgc atc gtg ttc acc gtg ctg agc atc gtg aac cgc gtg     2016
Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
            660                 665                 670

cgc cag gga tgc agc ccc ctg agc ttc cag acc cgc ctg ccc gtg tga     2064
Arg Gln Gly Cys Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val  *
        675                 680                 685

cgg atc c                                                           2071
Arg Ile
```

<210> 70
<211> 689
<212> PRT
<213> Artificial Sequence

<400> 70

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Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
```

```
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620
Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
625                 630                 635                 640
Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
                645                 650                 655
Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
            660                 665                 670
Arg Gln Gly Cys Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val Arg
        675                 680                 685
Ile
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<210> 71
<211> 2469

<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(2469)

<400> 71

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gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc        48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag        96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg       144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag       192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60

aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat       240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc       288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac       336
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

acc aac aac acc cgc tgg ggc acc cag gag atg aag aac tgc agc ttc       384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg       432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac       480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag       528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc       576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc       624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
```

```
acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg      672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg      720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240

atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300

aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc     1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag     1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag     1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc     1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg     1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc     1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430

cgc ccc ggc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac     1344
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
```

```
          435                 440                 445

aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag      1392
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460

gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc gtg ggc atc ggc      1440
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480

gct atg ttc ctc ggc ttc ctg ggc gct gca ggc agc acc atg ggc gcc      1488
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495

gcc agc ctg acc ctg acc gtg cag gcc cgc cag ctg ctg agc ggc atc      1536
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510

gtg cag cag cag aac aac ctg ctg cgc gcc atc gag gcc cag cag cac      1584
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525

ctg ctc cag ctg acc gtg tgg ggc atc aag cag ctc cag gcc cgc gtg      1632
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540

ctg gct cta gag cgc tac ctc cag gac cag cgc ttc ctg ggc atg tgg      1680
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560

ggc tgc tcc ggc aag ctg atc tgc acc acg gcc gtg ccc tgg aac gcc      1728
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575

agc tgg agc aac aag aac ctg agc cag att tgg gac aac atg acc tgg      1776
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590

atg gag tgg gag cgc gag atc agc aac tac acc gag atc atc tac agc      1824
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605

ctg atc gag gag agc cag aac cag cag gag aag aac gag ctg gac ctg      1872
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620

ctc cag ctg gac aag tgg gca agc ttg tgg aac tgg ttc aac atc acc      1920
Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
625                 630                 635                 640

aac tgg ctg tgg tac atc aag att ttc atc atg atc gtg ggc ggc ctg      1968
Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
                645                 650                 655

atc ggc ctg cgc atc gtg ttc acc gtg ctg agc atc gtg aac cgc gtg      2016
Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
            660                 665                 670

cgc cag ggc tac agc ccc ctg agc ttc cag acc cgc ctg ccc gtg ccc      2064
```

```
Arg Gln Gly Tyr Ser Pro Leu Ser Phe Gln Thr Arg Leu Pro Val Pro
        675                 680                 685

cgc ggc ccc gac cgc ccc gag ggc atc gag gag gag ggc ggc gag cgc      2112
Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu Arg
    690                 695                 700

gac cgc gac cgc agc acc cgc ctg gtg acc ggc ttc ctg ccc ctg atc      2160
Asp Arg Asp Arg Ser Thr Arg Leu Val Thr Gly Phe Leu Pro Leu Ile
705                 710                 715                 720

tgg gac gac ctg cgc agc ctg ttc ctg ttc agc tac cat cga ttg cgc      2208
Trp Asp Asp Leu Arg Ser Leu Phe Leu Phe Ser Tyr His Arg Leu Arg
                725                 730                 735

gac ctg ctg ctg atc gtg gcc cgc atc gtg gag ctg ctg ggc cgg cgc      2256
Asp Leu Leu Leu Ile Val Ala Arg Ile Val Glu Leu Leu Gly Arg Arg
            740                 745                 750

ggc tgg gag atc ctg aag tac tgg tgg aac ctg ctc cag tac tgg agc      2304
Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu Leu Gln Tyr Trp Ser
        755                 760                 765

cag gag ctg aag aac tct gca gtg agc ctg ctg aac gcc acc gcc atc      2352
Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn Ala Thr Ala Ile
    770                 775                 780

gcc gtg gcc gag ggc acc gac cgc gtg atc gag gtg gtg cag cgc atc      2400
Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val Val Gln Arg Ile
785                 790                 795                 800

tgg cgc ggc atc ctg cac atc ccc acc cga att cgc cag ggc ttc gag      2448
Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile Arg Gln Gly Phe Glu
                805                 810                 815

cgc gcc ctg ctg taa gga tcc                                         2469
Arg Ala Leu Leu  *  Gly Ser
            820
```

<210> 72
<211> 822
<212> PRT
<213> Artificial Sequence

<400> 72

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
```

```
                100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
            115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala Val Gly Ile Gly
465                 470                 475                 480
Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala
                485                 490                 495
Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln Leu Leu Ser Gly Ile
            500                 505                 510
Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His
        515                 520                 525
Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val
    530                 535                 540
Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg Phe Leu Gly Met Trp
545                 550                 555                 560
Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ala
                565                 570                 575
```

```
Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp Asp Asn Met Thr Trp
            580                 585                 590
Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr Glu Ile Ile Tyr Ser
        595                 600                 605
Leu Ile Glu Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Leu Asp Leu
    610                 615                 620
Leu Gln Leu Asp Lys Trp Ala Ser Leu Trp Asn Trp Phe Asn Ile Thr
625                 630                 635                 640
Asn Trp Leu Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu
                645                 650                 655
Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser Ile Val Asn Arg Val
            660                 665                 670
~~Arg~~ Gln Gly Tyr Ser Pro Leu Ser ~~Phe Gln~~ Thr Arg Leu Pro Val Pro
        675                 680                 685
Arg Gly Pro Asp Arg Pro Glu Gly Ile Glu Glu Glu Gly Gly Glu Arg
    690                 695                 700
Asp Arg Asp Arg Ser Thr Arg Leu Val Thr Gly Phe Leu Pro Leu Ile
705                 710                 715                 720
Trp Asp Asp Leu Arg Ser Leu Phe Leu Phe Ser Tyr His Arg Leu Arg
                725                 730                 735
Asp Leu Leu Leu Ile Val Ala Arg Ile Val Glu Leu Leu Gly Arg Arg
            740                 745                 750
Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu Leu Gln Tyr Trp Ser
        755                 760                 765
Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn Ala Thr Ala Ile
    770                 775                 780
Ala Val Ala Glu Gly Thr Asp Arg Val Ile Glu Val Val Gln Arg Ile
785                 790                 795                 800
Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile Arg Gln Gly Phe Glu
                805                 810                 815
Arg Ala Leu Leu Gly Ser
            820
```

<210> 73
<211> 1431
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(1431)

<400> 73

```
gct agc gcg gcc gac cgc ctg tgg gtg acc gtg tac tac ggc gtg ccc        48
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15

gtg tgg aag gac gcc acc acc acc ctg ttc tgc gcc agc gac gcc aag        96
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30

gcc tac gac acc gag gtg cac aac gtg tgg gcc acc cac gcg tgc gtg       144
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45

ccc acc gac ccc aac ccc cag gag gtg gtg ctg ggc aac gtg acc gag       192
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
    50                  55                  60
```

```
aac ttc aac atg ggc aag aac aac atg gtg gag cag atg cac gag gat      240
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
 65                  70                  75                  80

atc atc agc ctg tgg gac cag agc ctg aag ccc tgc gtg aag ctg acc      288
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                 85                  90                  95

ccc ctg tgc gtg acc ctg aac tgc acc aag ctg aag aac agc acc gac      336
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110

~~acc~~ aac aac acc cgc tgg ggc acc ~~cag~~ gag atg aag aac tgc agc ttc      384
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125

aac atc agc acc agc gtg cgc aac aag atg aag cgc gag tac gcc ctg      432
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140

ttc tac agc ctg gac atc gtg ccc atc gac aac gac aac acc agc tac      480
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160

cgc ctg cgc agc tgc aac aca tcg atc atc acc cag gcc tgc ccc aag      528
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175

gtg agc ttc gag ccc atc ccc atc cac ttc tgc gcc ccc gcc ggc ttc      576
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190

gcc atc ctg aag tgc aac aac aag acc ttc aac ggc acc ggc ccc tgc      624
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205

acc aac gtg agc acc gtg cag tgc acc cac gga att cgc ccc gtg gtg      672
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220

agc acc cag ctg ctg ctg aac ggc agc ctg gcc gag gag gag gtg gtg      720
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240

atc aga tct gag aac ttc acc aac aac gcc aag acc atc atc gtg cag      768
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255

ctg aac gag agc gtg gag atc aac tgc acc cgc ccc aac aac aac acc      816
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270

cgc aag agc atc cac atc ggc cct ggc cgc gcc ttc tac acc acc ggc      864
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285

gac atc atc ggc gac atc cgc cag gcc cac tgc aac atc tct aga acc      912
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
```

```
aac tgg acc aac acc ctg aag cgc gtg gcc gag aag ctg cgc gag aag      960
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320

ttc aac aac acc acc atc gtg ttc aac cag agc tcc ggc ggc gac ccc     1008
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335

gag atc gtg atg cac agc ttc aac tgc ggc ggc gag ttc ttc tac tgc     1056
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350

aac acc acc cag ctg ttc aac agc acc tgg aac gag acc aac agc gag     1104
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365

ggc aac atc act agt ggc acc atc acc ctg ccc tgc cgc atc aag cag     1152
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380

atc atc aac atg tgg cag gag gtg ggc aag gcc atg tac gcc ccc ccc     1200
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400

atc ggc ggc cag atc aag tgc ctg agc aac atc acc ggc ctg ctg ctg     1248
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415

acc cgc gac ggc ggc agc gac aac tcg agc agc ggc aag gag att ttc     1296
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430

cgc ccc ggc ggc ggc gac atg cgc gac aac tgg cgc agc gag ctg tac     1344
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445

aag tac aag gtg gtg aag atc gag ccc ctg ggc atc gcc ccc acc aag     1392
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460

gcc aag cgc cgc gtg gtg cag cgc gag aag cgc gcc tag                 1431
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala  *
465                 470                 475
```

<210> 74
<211> 476
<212> PRT
<213> Artificial Sequence

<400> 74

```
Ala Ser Ala Ala Asp Arg Leu Trp Val Thr Val Tyr Tyr Gly Val Pro
 1               5                  10                  15
Val Trp Lys Asp Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
            20                  25                  30
Ala Tyr Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val
        35                  40                  45
Pro Thr Asp Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu
```

```
    50                  55                  60
Asn Phe Asn Met Gly Lys Asn Asn Met Val Glu Gln Met His Glu Asp
65                  70                  75                  80
Ile Ile Ser Leu Trp Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr
                85                  90                  95
Pro Leu Cys Val Thr Leu Asn Cys Thr Lys Leu Lys Asn Ser Thr Asp
            100                 105                 110
Thr Asn Asn Thr Arg Trp Gly Thr Gln Glu Met Lys Asn Cys Ser Phe
        115                 120                 125
Asn Ile Ser Thr Ser Val Arg Asn Lys Met Lys Arg Glu Tyr Ala Leu
    130                 135                 140
Phe Tyr Ser Leu Asp Ile Val Pro Ile Asp Asn Asp Asn Thr Ser Tyr
145                 150                 155                 160
Arg Leu Arg Ser Cys Asn Thr Ser Ile Ile Thr Gln Ala Cys Pro Lys
                165                 170                 175
Val Ser Phe Glu Pro Ile Pro Ile His Phe Cys Ala Pro Ala Gly Phe
            180                 185                 190
Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys
        195                 200                 205
Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val
    210                 215                 220
Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val
225                 230                 235                 240
Ile Arg Ser Glu Asn Phe Thr Asn Asn Ala Lys Thr Ile Ile Val Gln
                245                 250                 255
Leu Asn Glu Ser Val Glu Ile Asn Cys Thr Arg Pro Asn Asn Asn Thr
            260                 265                 270
Arg Lys Ser Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Gly
        275                 280                 285
Asp Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg Thr
    290                 295                 300
Asn Trp Thr Asn Thr Leu Lys Arg Val Ala Glu Lys Leu Arg Glu Lys
305                 310                 315                 320
Phe Asn Asn Thr Thr Ile Val Phe Asn Gln Ser Ser Gly Gly Asp Pro
                325                 330                 335
Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
            340                 345                 350
Asn Thr Thr Gln Leu Phe Asn Ser Thr Trp Asn Glu Thr Asn Ser Glu
        355                 360                 365
Gly Asn Ile Thr Ser Gly Thr Ile Thr Leu Pro Cys Arg Ile Lys Gln
    370                 375                 380
Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro
385                 390                 395                 400
Ile Gly Gly Gln Ile Lys Cys Leu Ser Asn Ile Thr Gly Leu Leu Leu
                405                 410                 415
Thr Arg Asp Gly Gly Ser Asp Asn Ser Ser Ser Gly Lys Glu Ile Phe
            420                 425                 430
Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu Tyr
        435                 440                 445
Lys Tyr Lys Val Val Lys Ile Glu Pro Leu Gly Ile Ala Pro Thr Lys
    450                 455                 460
Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg Ala
465                 470                 475
```

<210> 75
<211> 1038
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)...(1038)

<400> 75

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gcc gtg ggc atc ggc gct atg ttc ctc ggc ttc ctg ggc gct gca ggc        48
Ala Val Gly Ile Gly Ala Met Phe Leu Gly Phe Leu Gly Ala Ala Gly
 1               5                  10                  15

agc acc atg ggc gcc gcc agc ctg acc ctg acc gtg cag gcc cgc cag        96
Ser Thr Met Gly Ala Ala Ser Leu Thr Leu Thr Val Gln Ala Arg Gln
            20                  25                  30

ctg ctg agc ggc atc gtg cag cag cag aac aac ctg ctg cgc gcc atc       144
Leu Leu Ser Gly Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile
        35                  40                  45

gag gcc cag cag cac ctg ctc cag ctg acc gtg tgg ggc atc aag cag       192
Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln
    50                  55                  60

ctc cag gcc cgc gtg ctg gct cta gag cgc tac ctc cag gac cag cgc       240
Leu Gln Ala Arg Val Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg
65                  70                  75                  80

ttc ctg ggc atg tgg ggc tgc tcc ggc aag ctg atc tgc acc acg gcc       288
Phe Leu Gly Met Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala
                85                  90                  95

gtg ccc tgg aac gcc agc tgg agc aac aag aac ctg agc cag att tgg       336
Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp
            100                 105                 110

gac aac atg acc tgg atg gag tgg gag cgc gag atc agc aac tac acc       384
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Ile Val Gly Gly Leu Ile Gly Leu Arg Ile Val Phe Thr Val Leu Ser
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        35                  40                  45
Glu Ala Gln Gln His Leu Leu Gln Leu Thr Val Trp Gly Ile Lys Gln
    50                  55                  60
Leu Gln Ala Arg Val Leu Ala Leu Glu Arg Tyr Leu Gln Asp Gln Arg
65                  70                  75                  80
Phe Leu Gly Met Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ala
                85                  90                  95
Val Pro Trp Asn Ala Ser Trp Ser Asn Lys Asn Leu Ser Gln Ile Trp
            100                 105                 110
Asp Asn Met Thr Trp Met Glu Trp Glu Arg Glu Ile Ser Asn Tyr Thr
        115                 120                 125
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225                 230                 235                 240
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Tyr His Arg Leu Arg Asp Leu Leu Leu Ile Val Ala Arg Ile Val Glu
            260                 265                 270
Leu Leu Gly Arg Arg Gly Trp Glu Ile Leu Lys Tyr Trp Trp Asn Leu
        275                 280                 285
Leu Gln Tyr Trp Ser Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu
    290                 295                 300
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305                 310                 315                 320
Val Val Gln Arg Ile Trp Arg Gly Ile Leu His Ile Pro Thr Arg Ile
                325                 330                 335
Arg Gln Gly Phe Glu Arg Ala Leu Leu
            340                 345
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A3

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(71) Applicant *(for all designated States except US)*: STATENS SERUM INSTITUT [DK/DK]; Artillerivej 5, DK–2300 Copenhagen S (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: FOMSGAARD, Anders [DK/DK]; Hostrups Have 5, DK–1954 Frederiksberg C (DK).

(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK–1021 Copenhagen K (DK).

(81) Designated States: AE, AG, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, DZ, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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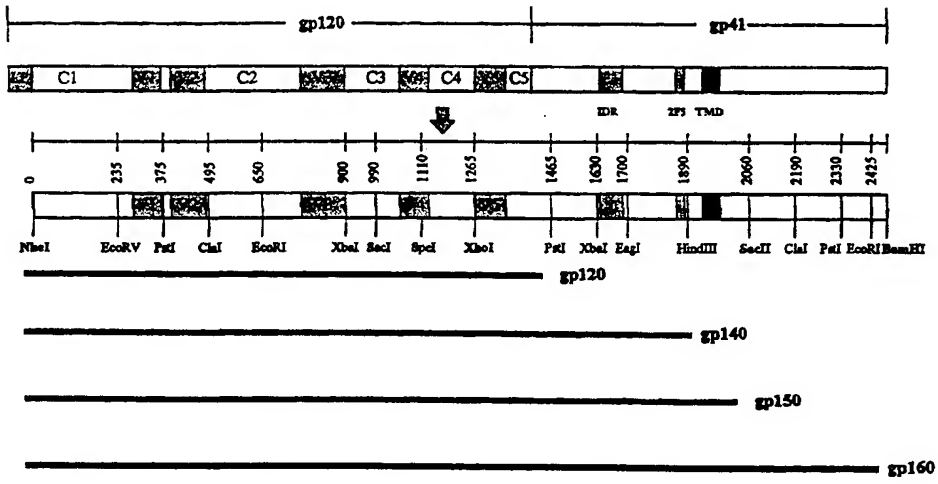
16 November 2000 (16.11.00)

(54) **Title:** NUCLEOTIDE CONSTRUCT WITH OPTIMISED CODONS FOR AN HIV GENETIC VACCINE BASED ON A PRIMARY, EARLY HIV ISOLATE AND SYNTHETIC ENVELOPE

Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms



(57) **Abstract**

The present invention relates to a method for producing a nucleotide sequence construct with optimized codons for an HIV genetic vaccine based on a primary, early HIV isolate. Specific such nucleotide sequence construct are the synthetic envelope BX08 constructs. The invention further relates to the medical use of such constructs for the treatment and prophylaxis of HIV through DNA vaccine and for diagnostics.

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/DK 00/00144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/16 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2000	14/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	ZOLLA-PAZNER S. ET AL.: "Neutralization of syncytium-inducing primary isolates by sera from human immunodeficiency virus (HIV)-uninfected recipients of candidate HIV vaccines." J. INFECT. DIS., vol. 178, no. 5, November 1998 (1998-11), pages 1502-1506, XP000857047 abstract	1-61
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A	WO 97 48370 A (DAVIES MARY ELLEN ;PERRY HELEN C (US); SHIVER JOHN W (US); FREED D) 24 December 1997 (1997-12-24) abstract	1-61
A	WO 98 12207 A (GEN HOSPITAL CORP) 26 March 1998 (1998-03-26) abstract figure 2	1-61

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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	HAAS J ET AL: "CODON USAGE LIMITATION IN THE EXPRESSION OF HIV-1 ENVELOPE GLYCOPROTEIN" CURRENT BIOLOGY,GB,CURRENT SCIENCE,, vol. 6, no. 3, page 315-324 XP000619599 ISSN: 0960-9822 cited in the application the whole document	1-61
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A	VERRIER F.C. ET AL.: "Antibodies to several conformation-dependent epitopes of gp120/gp41 inhibit CCR-5-dependent cell-to-cell fusion mediated by the native envelope glycoprotein of a primary macrophage-tropic HIV-1 isolate" PROC. NATL. ACAD. SCI. USA, vol. 94, August 1997 (1997-08), pages 9326-9331, XP002123320 cited in the application the whole document	1-58
A	WO 98 34640 A (DAVIES MARY ELLEN M ;PERRY HELEN C (US); FREED DANIEL C (US); LIU) 13 August 1998 (1998-08-13) abstract	3
A	GAO F. ET AL.: "Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID networks for HIV isolation and characterization" J. VIROL., vol. 70, no. 3, March 1996 (1996-03), pages 1651-1667, XP002123321 the whole document	4-7
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Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 98 01570 A (UNIV VANDERBILT ;MITCHELL WILLIAM M (US)) 15 January 1998 (1998-01-15) abstract figure 7	44-49

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/DK 00/00144

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KIENY M P ET AL: "IMPROVED ANTIGENICITY OF THE HIV ENV PROTEIN BY CLEAVAGE SITE REMOVAL" PROTEIN ENGINEERING,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 2, no. 3, page 219-225 XP000000860 ISSN: 0269-2139 the whole document ---	44-49
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Information on patent family members

Inter ›nal Application No
PCT/DK 00/00144

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(71) Applicant *(for all designated States except US)*: STATENS SERUM INSTITUT [DK/DK]; Artillerivej 5, DK–2300 Copenhagen S (DK).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: FOMSGAARD, Anders [DK/DK]; Hostrups Have 5, DK–1954 Frederiksberg C (DK).

(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK–1021 Copenhagen K (DK).

(81) Designated States: AE, AG, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, DZ, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

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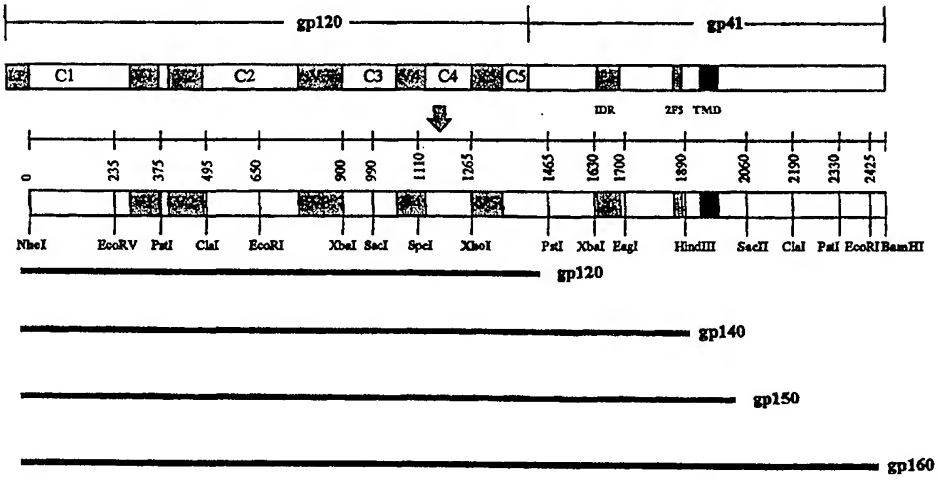
Without international search report and to be republished upon receipt of that report.
Without classification; title and abstract not checked by the International Searching Authority.

(54) **Title:** METHOD FOR PRODUCING A NUCLEOTIDE SEQUENCE CONSTRUCT WITH OPTIMISED CODONS FOR AN HIV GENETIC VACCINE BASED ON A PRIMARY, EARLY HIV ISOLATE AND SYNTHETIC ENVELOPE BX08 CONSTRUCTS

Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms



(57) **Abstract**

The present invention relates to a method for producing a nucleotide sequence construct with optimized codons for an HIV genetic vaccine based on a primary, early HIV isolate. Specific such nucleotide sequence construct are the synthetic envelope BX08 constructs. The invention further relates to the medical use of such constructs for the treatment and prophylaxis of HIV through DNA vaccine and for diagnostics.

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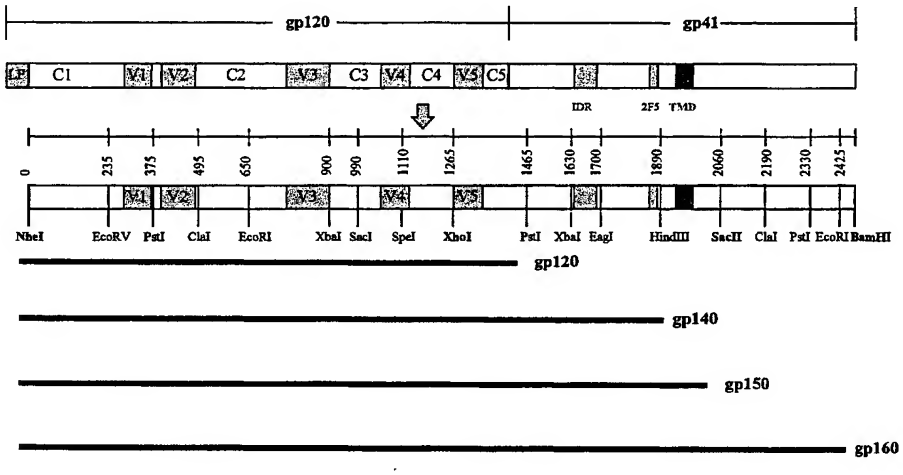
[Continued on next page]

(54) Title: NUCLEOTIDE CONSTRUCT WITH OPTIMISED CODONS FOR AN HIV GENETIC VACCINE BASED ON A PRIMARY, EARLY HIV ISOLATE AND SYNTHETIC ENVELOPE

Synthetic BX08 Env
Strategy for building the full-length gp160 and derived truncated forms



(57) Abstract: The present invention relates to a method for producing a nucleotide sequence construct with optimized codons for an HIV genetic vaccine based on a primary, early HIV isolate. Specific such nucleotide sequence construct are the synthetic envelope BX08 constructs. The invention further relates to the medical use of such constructs for the treatment and prophylaxis of HIV through DNA vaccine and for diagnostics.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 00/00144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/16 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VINNER L ET AL: "Gene gun DNA vaccination with Rev-independent synthetic HIV-1 gp160 envelope gene using mammalian codons" VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 17, no. 17, 23 April 1999 (1999-04-23), pages 2166-2175, XP004165002 ISSN: 0264-410X the whole document --- -/--	1-61

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
7 August 2000	11. 10. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 98 12207 A (GEN HOSPITAL CORP) 26 March 1998 (1998-03-26) abstract figure 2	1-61

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BACK N K T ET AL: "AN N-GLYCAN WITHIN THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 GP120 V3 LOOP AFFECTS VIRUS NEUTRALIZATION" VIROLOGY,US,ACADEMIC PRESS,ORLANDO, vol. 199, page 431-438 XP002910682 ISSN: 0042-6822 the whole document	40,41
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIENY M P ET AL: "IMPROVED ANTIGENICITY OF THE HIV ENV PROTEIN BY CLEAVAGE SITE REMOVAL" PROTEIN ENGINEERING,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 2, no. 3, page 219-225 XP000000860 ISSN: 0269-2139 the whole document ---	44-49
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00144

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 57 and 58 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
T/DK 00/00144

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